Sensory integration in the hippocampal formation of pre- and post-weanling rats

Laurenz Mussig

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Declaration

I, Laurenz Mussig, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Laurenz Mussig

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Abstract

'Place cells' are hippocampal pyramidal neurons which fire only when an animal visits a particular location in an environment ('place fields'). Their location-specific firing is supported by configurations of multi-modal sensory cues. Almost all work on place cells focused on the properties of these cells in adult rats. Recent work (Langston et al., 2010; Scott et al., 2011; Wills et al., 2010) however could identify place cells in the hippocampus of very young rat pups (ca. 2 weeks old), and furthermore show that these cells undergo a strong functional maturation in terms of their location-specific firing properties.

In this thesis, we investigated when the configural integration of sensory information first emerges during the postnatal development of the hippocampus, by probing the response of place cells to manipulations of sensory cues in a familiar environment in pre- (aged 2-3 weeks) and post-weanling (aged 3-4 weeks) rat pups as well as in adult controls. These included changing certain parts of the local olfactory/tactile cues as well as removing visual cues. Recordings were also undertaken in a completely novel environment. These experiments will further our understanding about how the brain's system for the representation of space develops and in particular will shed light on the question whether place cells in young rat pups are driven by single cues or already have configural properties as in adult rats.

The results described in this thesis are compatible with the view that place cell responses recorded in very young rat pups already integrate multimodal cues, as in adults, suggesting that hippocampal spatial responses are inherently configural. However, some evidence points out to a stronger influence of local non-visual intra-maze cues early in development, while the influence of vision seems to increase across development. This could be due to the concurrent maturation of the sensory systems. In a novel environment, animals of all ages form a novel

spatial representation, showing that even animals as old as 2 weeks can already distinguish between two distinct environments.

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I Introduction

From an ethological perspective the ability of rodents and other animals to navigate from a safe shelter to food sources and back is of major importance for the individual's survival. Behavioural studies with laboratory rats in the 1940's (Tolman et al., 1946a, 1946b) led Tolman to propose that the brain of these animals contains a system for the representation of space, that allows the formation of a map-like representation of an environment (Tolman, 1948). This would enable animals to react flexibly to changes in their surroundings like e.g. the use of short cuts or the use of novel alternative routes to a goal location.

After the discovery of place cells in the hippocampus of adult rats by O'Keefe and Dostrovsky (1971) in the early 1970's, the building blocks for this map-like representation in the mammalian brain seemed to have been identified. This discovery lead O'Keefe and Nadel (1978a) to propose their cognitive map theory: as the main function of individual pyramidal cells inside the hippocampus appeared to be the representation of different locations in an environment, O'Keefe and Nadel proposed that the hippocampal system contains a map-like representation of an environment. This representation was thought to be unique to a given environment and furthermore stable over time. Subsequent work gathered compelling evidence that place cells are not something specific to the rodent brain, but rather a general property of the hippocampus of vertebrates: place cells have also been described in pigeons (Bingman et al., 2006), bats (Ulanovsky and Moss, 2007; Yartsev and Ulanovsky, 2013) and even humans (Ekstrom et al., 2003).

By investigating the properties of place cells in more detail it quickly became clear that, rather than responding to simple sensory cues, these cells form a higher-order representation of space. However, a sufficiently large manipulation of sensory stimuli present in an environment can lead to a complete change in the pattern of place cell activity meaning that the same physical space can be represented by a different ensemble of place cells (Bostock et al., 1991; O'Keefe and Conway, 1978), a process called 'remapping'.

However, in contrast to the vast abundance of literature on place cell function in freely moving adult rats, only very little is known about how these cells behave/function in very young rat pups. In their proposal of the cognitive map theory O'Keefe and Nadel (1978a) argued that this system should be a synthetic *a priori* system, thus not requiring any behavioural experience. This is in line with a Kantian view of the perception of space, as Kant argued that organisms are born with an *a priori* knowledge of space and hence do not need to 'learn' this ability (Kant, 1781).

The work presented in this thesis builds on recent work (Langston et al., 2010; Scott et al., 2011; Wills et al., 2010) which showed that place cells can be identified in developing animals (ca. 2-4 weeks old), but that these cells undergo a strong functional maturation in terms of their firing properties. This already seems to indicate that the hippocampal representation of space is not a strict synthetic *a priori* system. Furthermore, these studies did not test whether place cells in these animals do already form a higher-order representation of space as in adult rats and more generally what sensory cues drive place cell firing in young animals. This thesis aims to shed light on exactly these properties of place cells in young rat pups, thus trying to elucidate exactly which sensory modalities are integrated at the place cell level in such animals, and whether these properties might also show an experience-dependent maturation. This thesis will mainly focus on the responses of place cells of pre-weanling rat pups (i.e. less than 3 weeks old) to sensory manipulations of a familiar environment. The reason for this focus will become clear throughout the introduction of this thesis: most sensory systems do not yet show adult-like properties at these ages and furthermore functional input from another class of spatially tuned neurons ('grid cells') to place cells is most probably still absent.

I.1 Anatomy of the hippocampal and parahippocampal cortices

I.1.1 Geometry of structures

In this chapter I will give an overview of the anatomy of the hippocampal (HF) and parahippocampal (PHF) formations and the interconnectivity of their parts in the laboratory rat, *rattus norvegicus*. Before going into detail it is important to clarify the geometrical logic I will follow and to give some definitions of terms that can be ambiguous in the literature. I will mainly follow the logic used by van Strien et al. (2009) and the book chapter by Amaral and Lavenex in the 'Hippocampus Book' (Amaral and Lavenex, 2007) (for an overview of hippocampal anatomy see Figure I-1).

- Hippocampal formation: consists of the dentate gyrus (DG), the hippocampus proper (HCP) which includes the CA subfields (CA1, CA2 and CA3), and the subiculum (SUB).
- Parahippocampal formation: consists of the pre- (PrS) and parasubiculum (PaS), the entorhinal cortex (EC) and the peri- (PER) and postrhinal (POR) cortices.

As far as the overall geometry of the hippocampal formation is concerned three axes are important to give a three-dimensional description of its parts:

- The septotemporal axis which is often referred to as the long axis describing the trajectory of the hippocampal formation as it bends from its septal end ventrally to its temporal pole.
- The transverse axis which runs perpendicular to the septotemporal axis and in parallel to the cell layers.

- The superficial-to-deep axis which is the axis electrodes will follow as they are lowered dorsoventrally during a typical electrophysiological experiment. This axis is running in opposite direction to the actual layer structure of HF since due to its inward bending the deep layers are actually lying more superficially inside the brain if one moves from dorsal to ventral (for CA1).

For the structures of PHF different types of axes are used. They all share the superficial to deep orientation from the pial surface to deeper regions. For pre- and parasubiculum there is a septotemporal and a proximodistal aspect as for regions in HF. The entorhinal cortex is characterised by a dorsomedial to ventrolateral axis and peri- and postrhinal cortices by a dorsoventral axis. Entorhinal, peri- and postrhinal cortices also all have a rostral to caudal gradient.

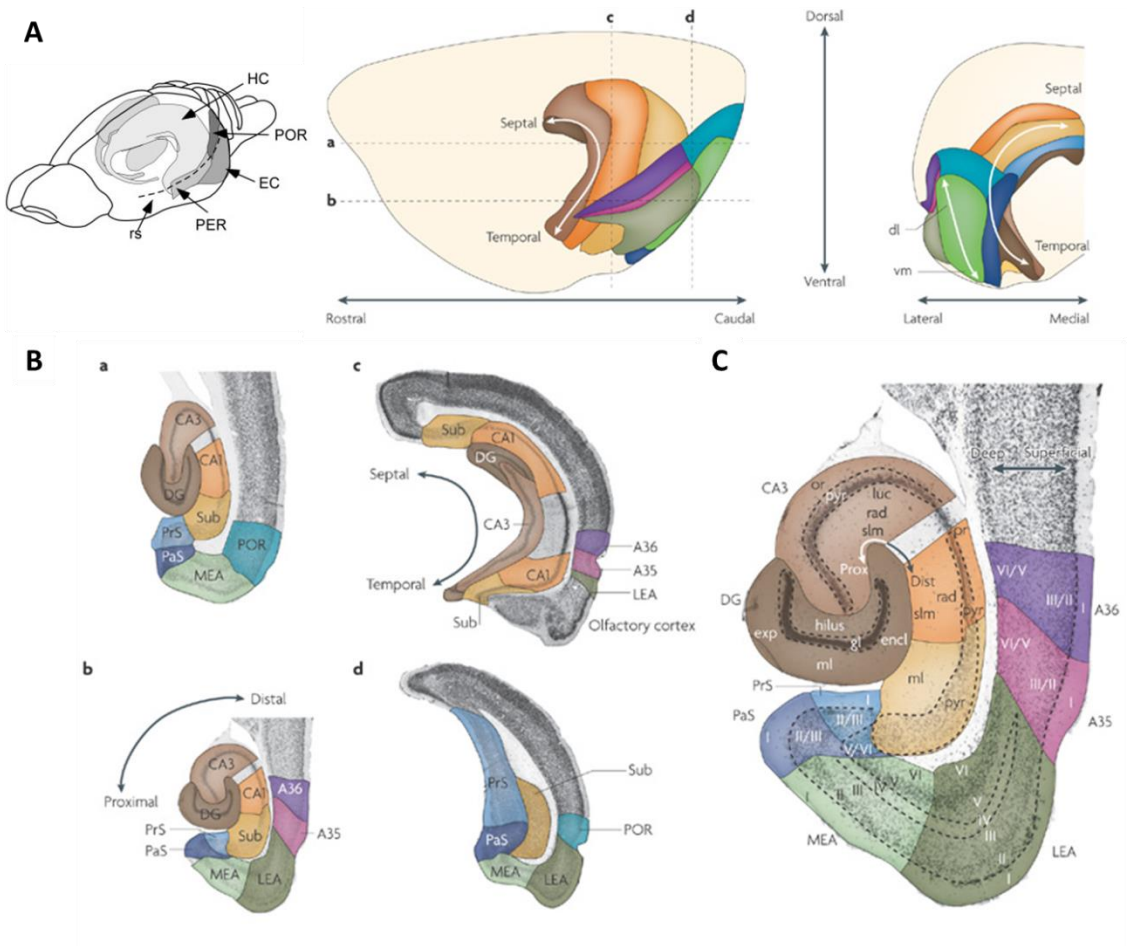


Figure I-1: Overview of hippocampo-parahippocampal anatomy. A: schematic overview of location of hippocampus (HC) and rhinal cortices (PER: perirhinal cortex; EC: entorhinal cortex; POR: postrhinal cortex; rs: rhinal sulcus) inside the rat brain (lateral view; leftmost panel). Middle and right panel indicate axes of hippocampal (septotemporal, transverse (dotted line at a) and superficial-to-deep (dorsal-ventral)) and parahippocampal (only for EC: dorsolateral (dl) to ventromedial (vm) axis (white arrow)) formation by showing a schematic lateral (middle) and caudal (right) view of rat brain with the respective structures (colours indicate structures from B and C). Dotted lines in middle panel indicate horizontal (a, b) and coronal (c, d) sections shown in B. B: a, b: horizontal sections from A. c, d: coronal sections from A. C: enlarged view of Bb. Roman numbers indicate cortical layers. DG: dentate gyrus (dark brown), CA3 (medium brown), CA1 (orange), Sub: subiculum (yellow ochre), PrS: presubiculum (light blue), PaS: parasubiculum (dark blue), MEA: medial entorhinal cortex (light green), LEA: lateral entorhinal cortex (dark green), A35, 36 (perirhinal cortex): area 35 (pink), 36 (purple), POR: postrhinal cortex (turquoise). Adapted from Furtak et al. (2007) (A, leftmost panel) and van Strien et al. (2009) (remaining figure).

I.1.2 General overview of anatomy and connectivity of hippocampal formation

HF in the rat is a C-shaped structure in each hemisphere of the brain which together vaguely resemble two bananas connected at their stems, with the connection being the corpus callosum. It lies in the more caudal part of the brain but generally spans a large portion of the more posterior portions of the rostrocaudal axis in the rat. As it is part of the so-called archi- or allocortex, it does not have the typical neocortical six-layer structure, but is instead comprised of three main layers: A deep polymorph layer, a principal cell layer and a superficial layer which is usually referred to as molecular layer.

The central pathway for the flow of information in HF is the tri- or polysynaptic circuit (Anderson et al., 1971) which is formed by the perforant pathway connecting entorhinal cortex with dentate gyrus (1^{st} synapse), the mossy fibres connecting dentate gyrus with the CA3 subfield of the hippocampus proper (2^{nd} synapse) and the Schaffer collaterals which connect CA3 to CA1 (3^{rd} synapse) (see Figure I-2). From CA1 the output from HF is sent via projections to the subiculum and back to entorhinal cortex. In the classical literature this circuit is often described as unidirectional. However, due to better tracing and imaging tools this view is thought to be an oversimplification, and it is now widely accepted that the real picture is much more complex. In the following sections I will describe the anatomy of each part of HF (see Figure I-1 and Figure I-3 for an overview) and their interconnectivity, and then go on to describe the anatomy of PHF and its connectivity with HF as well as its interconnectivity.

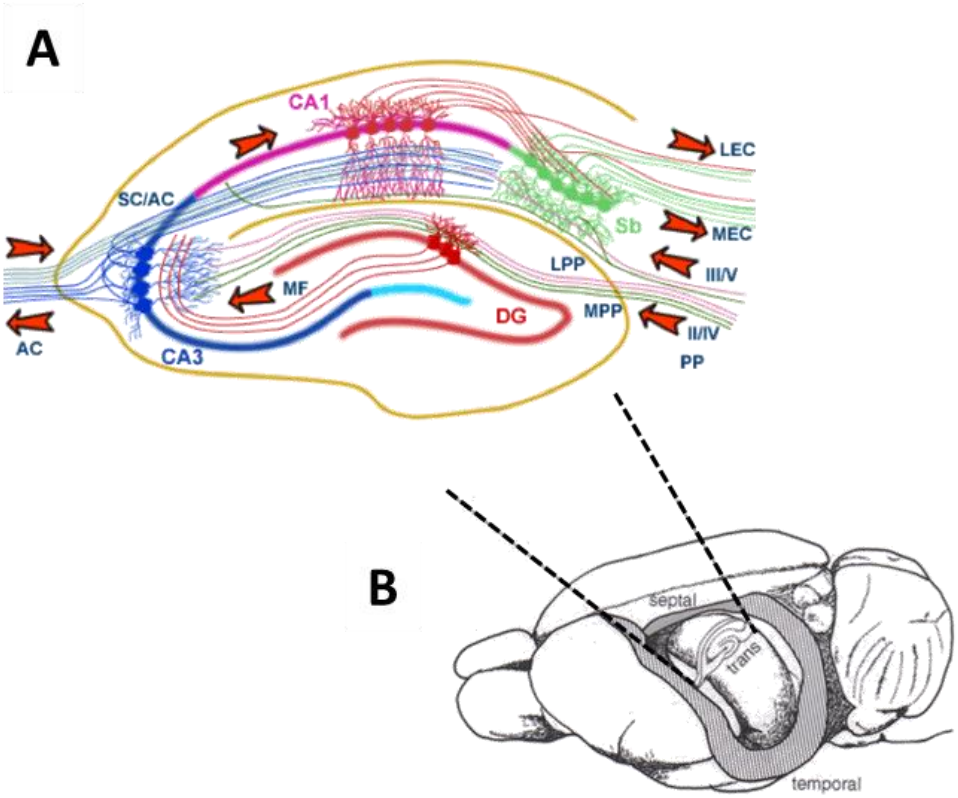


Figure I-2: Overview of general flow of information in hippocampus (trisynaptic circuit). A: schematic hippocampal slice indicating major connections and information flow (red arrows). The input comes from entorhinal cortex (EC – split into medial (MEC) and lateral (LEC) EC) and projects via the perforant path (PP – split into medial (MPP, dark green) and lateral (LPP, pink)) to dentate gyrus (DG, red) and CA3 (blue) as well as CA1 (pink) and subiculum (Sb, green). DG projects to CA3 via the mossy fibres (MF, red). CA3 projects to CA1 via Schaffer collaterals (SC) and to the contralateral hippocampus via the associational commissural pathway (AC). CA1 receives additional input from AC of contralateral side and projects to subiculum and EC. Roman numbers indicate EC layers. Adapted from www.bristol.ac.uk/synaptic/pathways. B: line drawing of rat brain (lateral view), indicating location of hippocampus and transverse slice shown in A (adapted from Amaral and Lavenex (2007)).

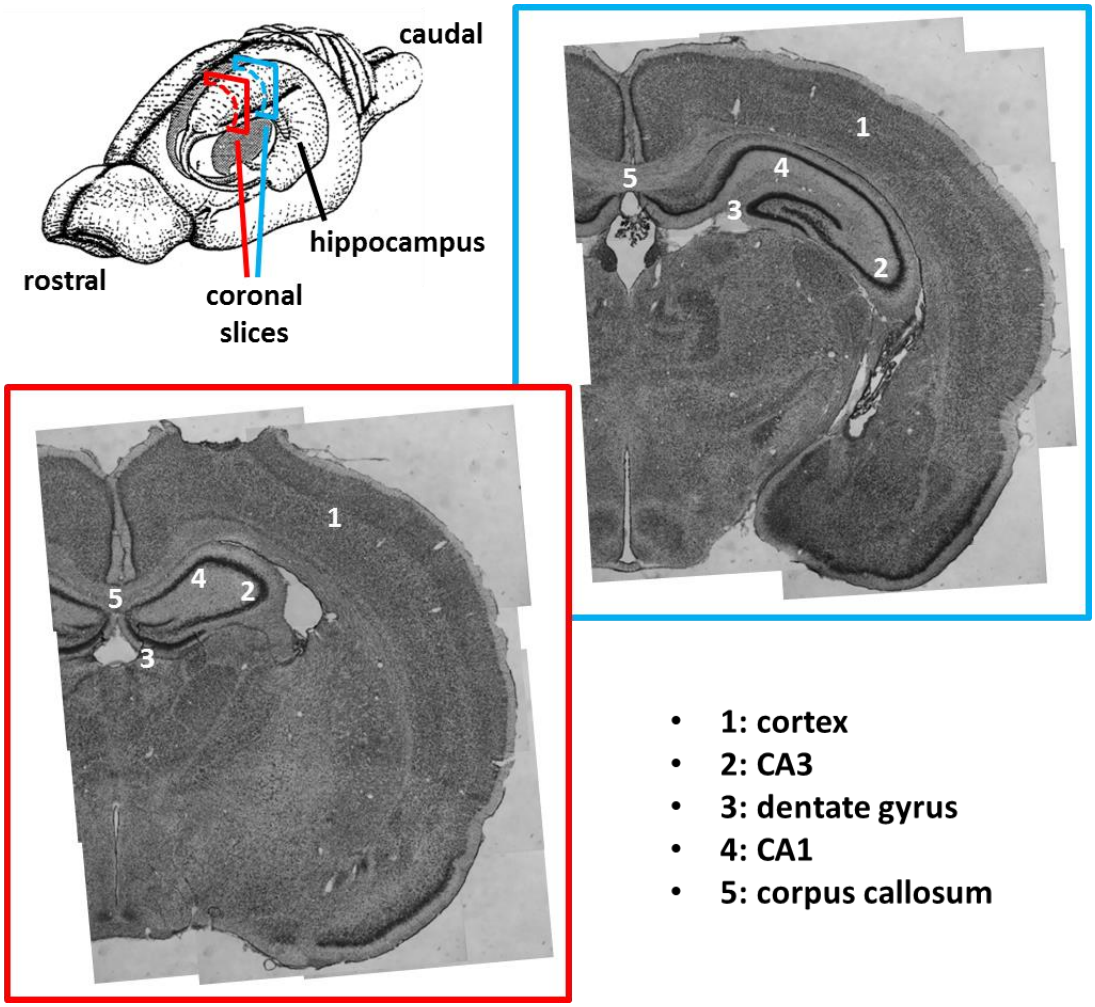


Figure I-3: Hippocampal anatomy. Top left shows line drawing of lateral view of rat brain with hippocampus (adapted from Amaral and Witter (1995)). Colour of coronal slices corresponds to boxes with coronal sections of rat brain (only left half of brain is shown in slices). Red box coronal slice is taken ca. 2.8 mm posterior to bregma and slice in blue box ca. 3.5 mm posterior to bregma. Numbers on slices indicate brain regions.

I.1.3 Dentate gyrus

I.1.3.1 Anatomy of DG

Dentate gyrus is a V- or U-shaped structure located at the proximal pole of the transverse axis of HF and comprised of a supra- and infra-pyramidal blade which encapsulate layer CA3 of the hippocampus proper in coronal and horizontal histological sections (see Figure I-3). The deep layer of DG is called the hilus and contains various afferent and efferent fibres as well as some interneurons. The most common one is the mossy cell whose dendrites usually stay within the hilus (Amaral, 1978). The main difference to other types of interneurons is that mossy cells are glutamatergic (Wenzel et al., 1997) and send axons to the molecular layer of the ipsi- and contralateral DG, thus possibly forming an associational network similar to the recurrent connections of CA3 (Ishizuka et al., 1990). Superficial to the hilus is the principal cell layer containing the somas of the principal cells which are called granule cells in DG. The dendritic trees of granule cells typically do not enter the hilus, but branch into the superficial molecular cell layer (Claiborne et al., 1990).

I.1.3.2 Afferents to DG

The main input to DG comes through the aforementioned perforant pathway from EC (Andersen et al., 1966a, 1966b; Ramon y Cajal, 1893; Steward and Scoville, 1976; Witter, 2007). The perforant pathway is divided into a lateral and a medial part referring to its origin in lateral (LEC) and medial (MEC) entorhinal cortex (Andersen et al., 1966a, 1966b). The majority of EC-to-DG projections arise from layer II (Steward and Scoville, 1976), but also neurons from

layers III, V and VI contribute to the input to DG (Köhler, 1985a; Steward and Scoville, 1976; Witter, 2007). A large proportion of the perforant projections form asymmetrical synapses onto granule cell dendrites (Matthews et al., 1976; Nafstad, 1967), and only a minor part targets interneurons (Zipp et al., 1989).

Apart from input originating in EC, DG also receives projections from other brain areas. There is a substantial subcortical input arising mainly from septal nuclei, i.e. the medial septum and the diagonal band of Broca (Amaral and Kurz, 1985a; Mosko et al., 1973). These projections mainly target cells in the hilar region of DG, in a zone just beneath the granule cell layer and are cholinergic or stain positive for γ-aminobutyric acid (GABA) (Köhler et al., 1984; Nyakas et al., 1987).

I.1.3.3 Efferents from DG

The main output from DG is transmitted via the mossy fibres, which are the axons of granule cells. They exclusively terminate in the CA3 subfield of HCP (Blackstad et al., 1970), forming large and complex en-passant presynaptic terminals on the proximal dendrites (in stratum lucidum) of CA3 pyramidal cells (the postsynaptic components on CA3 pyramidal cells are called thorny excrescences) (Chicurel and Harris, 1992). Each granule cell contacts on average ca. 15 principal cells in CA3 via its mossy fibres (Acsády et al., 1998). Mossy fibres usually span the full transverse axis of the hippocampus proper, while staying mainly at a constant septotemporal level (Blackstad et al., 1970).

I.1.4 Hippocampus proper

I.1.4.1 Anatomy of HCP

HCP contains the three CA subfields CA3, CA2 and CA1. Along the transverse axis CA3 is located close to DG (proximal) while CA2 (between CA3 and CA1) and CA1 are situated in progressively distal parts (see Figure I-1). As for DG, all subfields show a three-layer structure. The deepest layer is called stratum oriens and contains the basal dendrites of pyramidal cells, some of the CA3 commissural connections and Schaffer collaterals, as well as a variety of interneurons. Just deep to the stratum oriens lays the alveus which contains axons from pyramidal cells of the CA subfields passing on to the fimbria/fornix fibre bundle and providing a major output route from HCP. Superficial to stratum oriens is the principal cell layer, which contains the somata of principal cells and is thus called pyramidal cell layer. This layer is more tightly packed in CA1 than in CA3/CA2, and pyramidal cells in CA3/CA2 are on average larger than the ones in CA1. Also, pyramidal cells in CA3 are much more heterogeneous in terms of their dendritic length and distribution of their dendritic trees across the layers, compared to those in CA1 (Ishizuka et al., 1995; Pyapali et al., 1998). In CA3 just superficial to the pyramidal cell layer is the stratum lucidum, which contains the mossy fibres from DG (stratum lucidum is absent in CA2 and CA1). The molecular layer (the most superficial layer) of the CA subfields is further subdivided from deep to superficial into stratum radiatum (deep) and stratum lacunosum-moleculare. The former contains the apical dendrites of pyramidal cells as well as CA3 commissures and Schaffer collaterals. The latter consists of the apical tufts of the pyramidal dendrites and is also the zone where most of the direct entorhinal input to HCP terminates (Hjorth-Simonsen and Jeune, 1972). Both sub-layers contain a large variety of interneurons.

I.1.4.2 Afferents to HCP

The entorhinal input to CA3/CA2 follows a fairly similar topography as the EC projections to DG (Witter, 1993). LEC predominantly projects to superficial parts of stratum lacunosum-moleculare, while MEC projects mainly to deeper portions of this layer. Most of this direct input arises from layer II in EC (Steward and Scoville, 1976). EC input to CA1 originates mainly from layer III (sometimes referred to as the temporoammonic path) and the topography of these connections is quite different to the ones to CA3/CA2 (Steward and Scoville, 1976). LEC projects most heavily to distal parts of CA1 (close to SUB) and MEC projects to more proximal parts (close to CA2).

Besides this, the CA subfields also receive direct input from subcortical areas, mainly from septal nuclei, which tend to innervate CA3 much heavier than CA1, terminating mainly in stratum oriens (Mosko et al., 1973). There is a prominent hypothalamic projection from the supramammillary area to CA2 (Maglóczky et al., 1994) and thalamic projections from midline nuclei (especially nucleus reuniens) to stratum lacunosum-moleculare of CA1 (Herkenham, 1978). Furthermore, like DG, the CA subfields also receive projections from brain stem nuclei which are mainly noradrenergic and serotonergic (Swanson et al., 1987). The noradrenergic input terminates preferentially in stratum lucidum and stratum lacunosum-moleculare and is much more prominent than the serotonergic one, which is quite sparse. In general it seems that CA3 receives much stronger monoaminergic input than CA1 (Swanson et al., 1987).

I.1.4.3 Intrinsic connections of HCP

Because of the stronger relevance to this project an overview of the intrinsic connections of HCP is given, which was not done for DG. The main focus lies on the projections from CA3 to i) ipsilateral CA3 (associational), ii) contralateral CA3 (commissural) and iii) to CA1 (Schaffer collaterals). All these different types of projections are true collaterals of the same cells in CA3, indicating that they most likely convey very similar information to different parts of HCP.

The ipsilateral connections are also called associational connections, as they project onto other CA3 pyramidal cells on the ipsilateral side. They follow a broad kind of topography. Pyramidal cells in CA3 near the border to DG project to ipsilateral pyramidal cells close to DG, while cells located in medial parts to parts close to CA1 tend to project to much of the full extent of the transverse axis (Ishizuka et al., 1990).

The commissural projections to contralateral HCP follow a similar topography, projecting to homologous parts on the contralateral side (Blackstad, 1956). Both types of recurrent projections are much less prominent in CA1, where the recurrent loop seems to be restricted to the septotemporal level of the cell of origin (Van Groen and Wyss, 1990a).

Before describing the Schaffer collaterals, it is noteworthy that in contrast to the classical view, there are back projections from all parts of HCP. There is a projection from CA3 pyramidal cells to the hilus of DG (Laurberg, 1979; Li et al., 1994) and also a projection from CA1 back to CA3 has been reported (Amaral et al., 1991; Cenquizca and Swanson, 2007; Laurberg, 1979). This projection arises most likely from interneurons in strata radiatum and oriens in CA1, projecting back to homologous sublayers in CA3 (Van Strien et al., 2009).

The by far most studied intrinsic connection of HCP are the Schaffer collaterals, which are axons from CA3 pyramidal cells targeting pyramidal cells in CA1 (Ishizuka et al., 1990; Li et al.,

1994). They generally terminate onto the basal dendrites of CA1 pyramidal cells in stratum oriens and onto apical dendrites in stratum radiatum, exhibiting an intricate and complex topography. In general a CA3 pyramidal cell at any septotemporal level sends collaterals to roughly 2/3^{rds} of the CA1 septotemporal extent.

I.1.4.4 Efferents from HCP

The main output from HCP is comprised of projections from CA1 to the adjacent SUB and to EC. Fibres from CA1 mainly terminate in the deep parts of the molecular layer of SUB (Amaral et al., 1991). The output from CA1 to SUB is topographically organised in a manner that proximal parts of CA1 (close to CA3) project to distal parts of SUB (close to PrS), while the opposite is true for distal parts of CA1. Cells in between proximal and distal parts tend to project to medial parts of SUB. A projection from a single CA1 pyramidal cell can extent to roughly 1/3rd of the transverse and 1/3rd of the septotemporal axis of SUB (Tamamaki et al., 1987).

The main output from CA1 to EC is directed to deep layers of EC (Van Groen and Wyss, 1990a), but direct connections are also reported to more superficial layers (Cenquizca and Swanson, 2007). The connections between CA1 and EC are also topographically organised, such that cells located in the septotemporal aspect of CA1 project onto the dorsolateral to ventromedial axis of EC, and proximal parts of CA1 innervate predominantly cells in MEC while distal parts project most strongly to LEC (Naber et al., 2001).

I.1.5 Subiculum

I.1.5.1 Anatomy of SUB

Much less is known about the anatomy and function of SUB compared to other parts of HF. Also as an in-depth description of its parts and connectivity is beyond the scope of this thesis, the reader is referred to a comprehensive review by O'Mara et al. (2001). The subiculum is the main output structure of HF and lies adjacent to the distal end of CA1 in the transverse axis of the hippocampus. As DG and the CA subfields it has a three-layer structure which is continuous with the layers of the CA subfields. Its deep polymorph layer is not very well characterised and hardly any information on cell types and connectivity is currently available. The principal cell layer of SUB is continuous with the pyramidal cell layer of CA1 and marked by a sudden widening at the CA1/SUB border.

I.1.5.2 Connectivity of SUB

The CA1-to-SUB projections were already described earlier (see section I.1.4.4). SUB also receives input from a variety of subcortical structures including e.g. septal nuclei (Lopes da Silva et al., 1990) or the medial mammillary nucleus (Gonzalo-Ruiz et al., 1992).

SUB is the main output structure of HF, and its major efferent connection is to EC. The connections of SUB with EC are reciprocal and follow a similar topography as the projections from CA1 to EC (see section I.1.4.4). EC projections to SUB originate mainly in layer III and to a lesser extent in layer II, while the projections from SUB to EC terminate in the deep layers (IV-

VI) of EC, with the strongest projections terminating in layer IV (Köhler, 1985b; Steward and Scoville, 1976).

Other projections from SUB exist to PrS and PaS (Swanson and Cowan, 1977), as well as to cortical (e.g. retrosplenial cortex (Wyss and Van Groen, 1992)) and subcortical areas, like e.g. the lateral septum (Witter et al., 1990), mammillary nuclei (Witter et al., 1990), amygdala (Canteras and Swanson, 1992) and the nucleus accumbens (Witter and Groenewegen, 1990).

I.1.6 Interneurons of the hippocampal formation

The view that interneurons are merely dampening the neural activity of principal cells in the hippocampus has long been substituted by models where these neurons play an integral role in orchestrating and synchronising the activity of large populations of principal cells (Somogyi and Klausberger, 2005). As now more than 15 types of interneurons are identified in CA1 alone, a complete description of this cell population is beyond the scope of this thesis. A paper by Freund and Buzsáki (1996) provides a comprehensive review on this topic.

In brief, interneurons of HF are GABAergic and are usually morphologically characterised by the general targets of their axonal projections, i.e. e.g. somata (e.g. basket cells), specific dendritic segments (e.g. O-LM cells) or axon initial segments (e.g. axo-axonic cells). Another characteristic is which cell types are targeted: principal cells (see examples above) or other inhibitory interneurons (e.g. interneuron-selective inhibitory cells). Finally the expression of certain calcium-binding proteins like parvalbumin or neuropeptides like somatostatin can distinguish subgroups of interneurons. Besides these structural and connectional differences, local circuit neurons in HF can exhibit very intricate connection patterns with gap junction coupling between cells of the same class (e.g. basket cells) and highly divergent projections to

many principal cells. In addition, some GABAergic cells in HF project to the contralateral side (commissures; e.g. somatostatin-positive hilar neurons), the medial septum (e.g. hippocampal-septal cell) or receive projections from the medial septum, thus not being true local circuit neurons.

I.1.7 Overview of parahippocampal anatomy and connectivity

In this section I will give a rather short overview of the anatomy and connectivity of PHF, focusing mainly on EC. All subsequently described structures exhibit more or less strongly the classical six-layer structure of the isocortex.

I.1.7.1 Pre – and parasubiculum

Anatomy of PrS and PaS

The presubiculum (PrS) lies at the distal end of SUB, and its dorsal part is often referred to as the postsubiculum (PoS) (Van Groen and Wyss, 1990b). Presubicular principal cells are pyramidal cells and its deep layers are somewhat continuous with those of SUB on one side and the deep layers of EC on the other. The parasubiculum (PaS) lies adjacent to PrS, and its principal cells are pyramidal cells, too. Its deep layers are continuous with the ones from EC. Both structures show the six-layer structure of isocortical areas.

Connectivity of PrS and PaS

Both PrS and PaS have associational and commissural projections, the former especially directed at layer I and III of the contralateral side (Van Groen and Wyss, 1990c). In terms of their subcortical connections it is noteworthy that as far as HF is concerned, both structures share unique and reciprocal connections with anterior thalamic nuclei (Van Groen and Wyss, 1995). The input mainly arrives in layers I, II and IV, while return projections mostly originate from layer VI.

Both structures differ however in their connectivity with HF and EC (Caballero-Bleda and Witter, 1993; Köhler, 1985b). While PrS almost exclusively projects to layers I and III of MEC, PaS sends fibres to layer II of LEC and MEC. Another difference can be observed for the connections with HF. PrS sends only weak connections to all fields of HCP and DG. In contrast to this, PaS has fairly strong connections with the molecular layer of DG and sends weak projections to stratum lacunosum-moleculare of the CA subfields and the molecular layer of SUB.

I.1.7.2 Entorhinal cortex

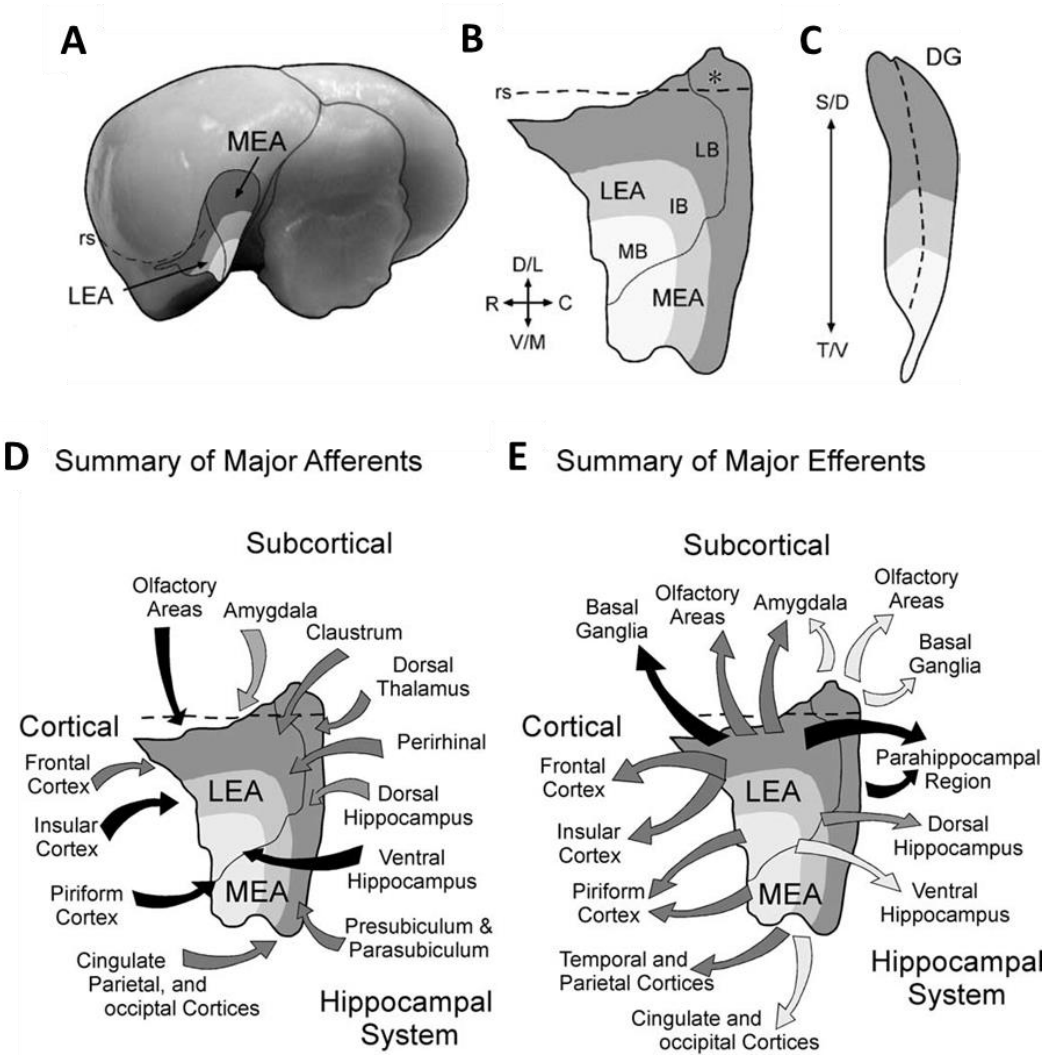


Figure I-4: Overview of band structure in EC as well as their interconnectivity with other areas. A: rat brain with location of medial (MEA) and lateral (LEA) entorhinal cortex (cerebellum is removed). rs: rhinal sulcus. B: unfolded map showing the bands in EC (LB: lateral band, IB: intermediate band, MB: medial band). Compass inset indicates axes (R-C: rostrocaudal, D/L-V/M: dorsolateral-ventromedial and asterisk indicates area where grid cells are predominantly found. C: unfolded map of dentate gyrus (DG). Colours correspond to entorhinal projections from different bands in B (S/D-T/V: septal/dorsal-temporal/ventral). D: summary of major afferents to EC. Strong connections are indicated by black arrows, moderate ones by dark grey arrows and weak ones by light grey arrows. E: summary of major efferents of EC with same arrow colour scheme as in D. Adapted from Kerr et al. (2007).

Anatomy of EC

In the rat EC is located in the most caudal, ventral and lateral part of the brain (see Figure I-4A) and is thought to be the main interface between HCP and the cortex. Generally, EC can be divided into LEC and MEC (Blackstad, 1956) with LEC occupying the rostrolateral and MEC the caudomedial part, resulting in a roughly triangular shape for both subdivisions. Dolorfo and Amaral (1998) suggested a different type of division of EC, based on the projections of EC fibres to DG which consist of three bands: a lateral, an intermediate and a medial band (see Figure I-4B, C). This will be explained in more detail below, after some remarks on the general cytoarchitecture of EC, which is described in great detail in a review by Canto, Wouterlood and Witter (2008).

EC shows the typical six-layer structure of the isocortex. Going from the pial surface ventrally, the first layer (layer I) is rather devoid of cells and contains mainly fibres oriented transversally.

Layer II is occupied by stellate and pyramidal cells, which both are often found in patches. Although this is more a feature of LEC, as the cells in MEC are larger and do not form clear 'cell islands'. Both cell types project to DG and CA3, with the main projections arising from stellate cells (Steward and Scoville, 1976; Tamamaki and Nojyo, 1993). Their dendritic trees usually branch only into layer I and II, and stellate cells are much more common in MEC than LEC (Canto et al., 2008; Klink and Alonso, 1997).

Layer III contains mainly pyramidal cells, but also a heterogeneous population of other cell types. The pyramidal cells send their projections to CA1 and SUB, but also send collaterals to layers I-III (Steward and Scoville, 1976; Witter et al., 1988).

Layer IV, the lamina dissecans, is predominantly cell-free, but does contain some interneurons and pyramidal cells.

Layer V is again a cellular layer which shows a band-like structure containing mainly three types of cells: Pyramidal cells, horizontal cells and polymorphic cells (Canto et al., 2008), all of which can be considered projection neurons (Hamam et al., 2002).

Finally, layer VI, lying just above the white matter, is not very well described so far, but contains multipolar cells and at least in MEC some pyramidal cells as well (Canto et al., 2008).

Connectivity of EC

A lot of the connections from EC to HF are reciprocal and were already partly described above (see sections I.1.4.4, I.1.5.2 and Figure I-4).

An interesting feature is the difference in the targets of projections arising from the three bands of LEC and MEC (Dolorfo and Amaral, 1998) (see Figure I-4B-E). The lateral band projects to the septal half of DG, the medial band to the temporal pole of DG, and the intermediate one to the portion of DG in between the projections of the lateral and medial band. But the more precise topography of the EC to HF and PHF input/output relations are even more intricate, and the reader is referred to a commentary on this matter by Kerr et al. (2007).

A special remark has to be made about the dorsocaudal part of MEC, the part where grid cells can be found (Hafting et al., 2005). This area receives more than 50% of its input from HF, almost exclusively arising from septal levels, with the input from temporal levels being weak (Kerr et al., 2007). It also receives a fairly strong input from the dorsal part of PrS (postsubiculum), an area where head direction cells can be found (Taube et al., 1990a).

EC is also connected with a large variety of other cortical (Agster and Burwell, 2009; Burwell and Amaral, 1998; Burwell, 2000; Insausti et al., 1997; Kerr et al., 2007) and subcortical structures (Kerr et al., 2007) (see Figure I-4D and E). Cortically directed efferents seem to

preferentially arise from deep layers (esp. layer V), while cortical input predominantly arrives in superficial layers I-III (Insausti et al., 1997; Swanson and Köhler, 1986). However, this view of a segregated neocortical-hippocampal-neocortical loop has been challenged by the observation that many pyramidal cells in layer V have dendrites branching into the superficial layers (Hamam et al., 2002, 2000), where they do receive excitatory input onto their dendrites (Lingenhöhl and Finch, 1991; Medinilla et al., 2013).

Strong additional cortical connections exist with the piriform cortex (LEC and MEC), insular regions (LEC) and occipital, parietal and frontal regions (mainly MEC). These connections also show some differences regarding the bands, as they are mostly uniformly distributed for LEC, and mainly target the intermediate and medial band in MEC (Kerr et al., 2007). For both LEC and MEC nearly all these connections are reciprocated.

Subcortical projections to LEC (about 1/3 of total input) include strong connections with olfactory areas (endopiriform nucleus and piriform transition area) and amygdala (esp. olfactory amygdala) (Pikkarainen et al., 1999). Particularly the connections with the olfactory areas are strongly reciprocated. MEC has similar subcortical connections, but in contrast to LEC, where the input is roughly equal for all bands, most projections terminate in the intermediate and medial band. Both LEC and MEC send significant amount of fibres to the basal ganglia, and for both the main thalamic input arises in dorsal thalamic nuclei, another region where head direction cells can be found (Taube, 1995).

I.1.7.3 Perirhinal and postrhinal cortices

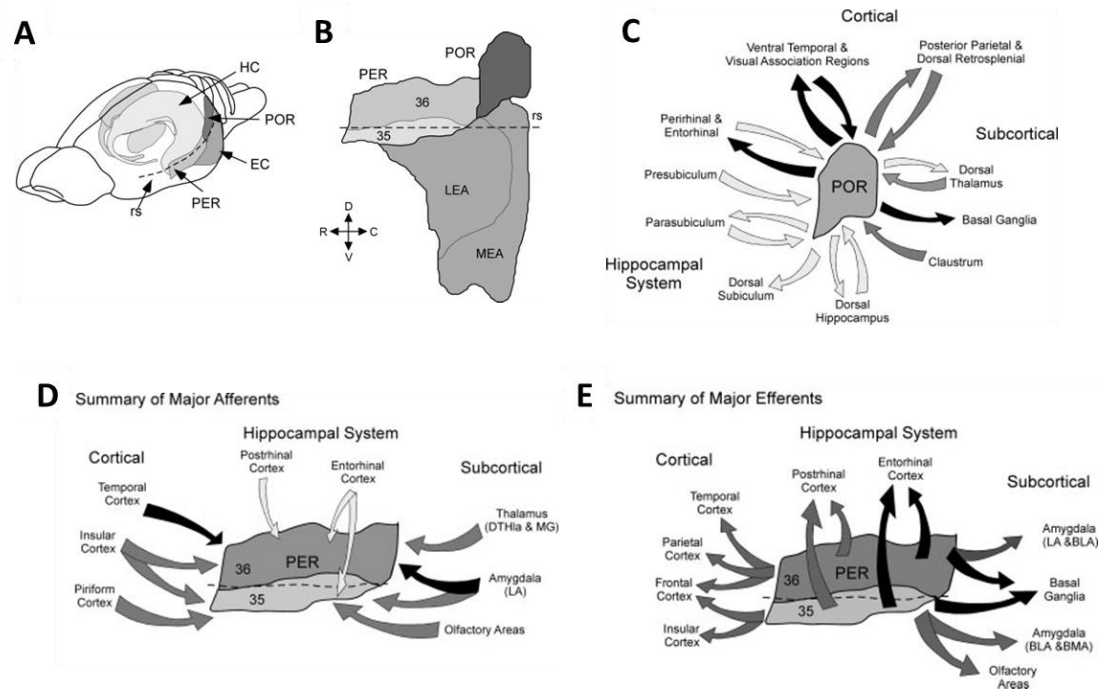


Figure I-5: Overview of connectivity of peri- and postrhinal cortices with other brain areas. A: schematic lateral view of the rat brain indicating location of rhinal cortices (EC: entorhinal cortex, HC: hippocampus, PER: perirhinal cortex, POR: postrhinal cortex, rs: rhinal sulcus). B: unfolded map of rhinal cortices. Area 35, 36 indicate different parts of PER. Compass inset indicates axes (D-V: dorsoventral, R-C: rostrocaudal). C: summary of major afferent and efferent connections to and from POR. Black arrows indicate strong connections, dark grey arrows indicate moderate ones and light grey arrows indicate weak ones. D: summary of major afferent connections to PER. Same arrow colour scheme as in C is applied. E: summary of major efferent connections from PER. Same arrow colour scheme as in C is applied. Adapted from Furtak et al. (2007).

A recent article from Rebecca Burwell's group gives a detailed description of the connectivity of PER and POR with HF, PHF and other regions of the brain (Furtak et al., 2007) (see Figure I-5).

The perirhinal and postrhinal cortices are located dorsal to EC. POR lies at the caudal pole of the brain, just dorsal to MEC and caudal to PER. PER, consisting of Brodmann area 35 and 36, is

situated rostral to POR along the rhinal sulcus (Burwell et al., 1995). In general afferents to POR are dominated by visuospatial input, while PER receives strong polymodal sensory projections (Furtak et al., 2007).

In terms of cortical connections, POR is strongly reciprocally connected with occipital regions, especially the lateral and medial visual association areas and the primary visual cortex. Roughly a quarter of the total input to POR arises from PHF and HF, with the strongest projections originating in PER, PaS and EC. These connections are largely reciprocated with a strong projection of POR to dorsocaudal MEC. In terms of HF the strongest (mostly afferent) connections of POR exist with the septal portion of CA1.

Cortical input to PER seems to be generally separated in olfactory input from the piriform cortex to area 35 and multimodal (auditory, visual and olfactory) sensory input to area 36 from the ventral temporal cortex.

I.1.8 Postnatal development of the hippocampal formation

In this section I will give an overview over the morphological and connectional development of the structures described in the previous section, but focusing mainly on HF.

I.1.8.1 General principles of hippocampal development

In general the morphological development of connections in the brain involves three steps: Axonal path finding, identification of target cells and synapse formation. In the hippocampus these processes are under the control of Cajal-Retzius cells (CR-cells) and reelin (Stanfield and Cowan, 1979), as well as components of the extracellular matrix (Förster et al., 1998). In addition, semaphorins, ephrins and neurotrophic factors play important roles as well (Skutella and Nitsch, 2001). Some general principles, with a few exceptions, seem to govern the structural development of HF (Bayer, 1980a). First, there is a rhinal to dentate gradient with areas closer to the rhinal sulcus maturing before areas further away. Second, there is superficial to deep gradient with deeper layers developing first. Third, fibres arriving earlier in a target zone tend project onto distal portions of the dendritic arbor, while later fibres usually target more proximal portions.

I.1.8.2 Anatomical development of the hippocampus proper

CA subfields

The stem cells for pyramidal and granule cells originate from the ventricular germinal layers at the hippocampal fissure. The peak of pyramidal cell proliferation occurs around embryonic day 16 and 17 (E16-17), with CA3 development slightly preceding the one of CA1 (peak CA3, E17; peak CA1, E18-19) (Bayer, 1980b). Thus, the CA subfield is one of the exemptions of the rhinal to dentate gradient as CA3 matures earlier. The development follows the typical inside-out process observed in the cortex (Frotscher, 1997), meaning that early formed cells inhabit the deeper layers and later formed ones the more superficial layers. The overall volume of HCP increases in spurts of growth between E15 and postnatal day 21 (P21), with most of the increase after E20 accounted for by the growth of apical and basal dendrites (Bayer, 1980a, 1980b). The growth of dendritic arborisation of CA1 continues up to P90 of adult life, is strongest between P5 and P15, and in particular the apical dendrite ramification (where EC fibres mainly project to) occurs between P15-24 (Pokorný and Yamamoto, 1981).

Dentate gyrus

The development of DG is somewhat special in the rat because at birth only 15% of the granule cells are already generated, and in general DG is the last structure to appear in HF (after E20) (Bayer, 1980b). Granule cell proliferation starts around E17 and continues well into postnatal life. By P5 roughly 50% of adult cell levels are reached and approximately 5-10 % are born after P18 (Bayer, 1980b). Dentate granule cells are therefore one of the few examples of postnatal neurogenesis which continues into adulthood. There are also other peculiarities in the

development of DG as the suprapyramidal blade development is earlier than the one of the infrapyramidal blade (Bayer, 1980b). By P7 both blades of DG are homogeneous. Furthermore, DG develops in an outside-in fashion, the opposite of the rest of HF.

I.1.8.3 Interneurons

There is still some debate about the exact locus of interneuron proliferation for HF in the embryonic brain, but there is good evidence that in contrast to pyramidal cells, the majority of interneurons originate from the subpallium of the telencephalon (the ventricular zone where pyramidal cells are formed is part of the more dorsal pallium) (reviewed in Danglot et al. (2006)).

Local interneuron proliferation in HF generally precedes the generation of principal cells and occurs between E13 and 18 (Amaral and Kurz, 1985b). However the exact layer position of interneurons can change up to ca. P15 (Jiang et al., 2001). By P5, GABAergic cells form synapses on pyramidal cells in HCP (Seress et al., 1989), although the length of their dendrites gradually increases until P20 (Lang and Frotscher, 1990). In this process CA1 lags slightly behind CA3. By P20, non-pyramidal cells in HCP exhibit all fine structures typical of adult cells (Lang and Frotscher, 1990).

I.1.8.4 Hippocampo-parahippocampal connectivity

By E22, EC shows its laminar structure and also PrS and PaS can be differentiated (Bayer, 1980a). In the mouse, fibres from EC reach HCP around E15 and DG around E18-19, and the first fibres from the medial septum arrive in HF around E17 (Supèr and Soriano, 1994). In the rat, at least a scaffold by CR-cell projections from stratum lacunosum-moleculare and the outer molecular layer of DG to EC is formed by E17, along which the EC projections will in-grow into HF (Ceranik et al., 1999). Commissures in CA3 start to be apparent around E18 and by P2 in DG in the mouse, so generally slightly later than the afferents from EC (Supèr and Soriano, 1994). Autoradiographical and degeneration studies in the rat by Loy et al. (1977) and Fricke & Cowan (1977) showed that by P3-4, EC projections show the adult-like laminar termination in DG, although it is not until P12 that a substantial number of spines on dentate granule cells can be observed.

Mossy fibres from granule cells are present in stratum lucidum of CA3 at P3, although the axons are still rather immature (Amaral and Dent, 1981). The typical postsynaptic component of the mossy fibre-to-pyramidal cell synapses (thorny excrescences) is not present until P9, although there is synaptic contact between these cells beforehand. By P21, mossy fibres seem to show most of the anatomical features of the adult rat (Amaral and Dent, 1981).

It generally seems to be true that perforant path projections and the associational/commissural fibre system are formed by the time of birth and undergo postnatal maturational processes like target cell contact and synapse formation (Loy et al., 1977).

I.1.9 Summary of hippocampal anatomy

In this section the anatomy of HF and PHF in terms of their respective neuron types and interconnectivity was highlighted. The general flow of information starts in layer II and III of EC and is directed at DG/CA3 and CA1 respectively. CA3 projects to CA1 and CA1 to SUB and the deep layers of EC, as does SUB, thereby closing the neocortical-hippocampal-neocortical loop. From deep layers of EC associational fibres project to more superficial layers as well as to other cortical regions. Furthermore the topographical organisation of the feedback projections in this system is often point-to-point reciprocal, i.e. areas that project to a certain region usually receive feedback projections from that very area. It is clear now that the view of the classical unidirectional signalling pathway through HF is an oversimplification, as at nearly all levels there are indeed back-projections to anterograde areas. The role of POR and PER as providers of multimodal sensory and visuospatial information to the hippocampo-parahippocampal system was also emphasised.

In the developmental section it is stressed that the connectivity of the hippocampo-entorhinal system is far from being mature at birth. Generally by P21 most of it shows properties of the adult rat, although certain parts, like e.g. the dendritic arborisation of CA1 neurons, are not completed until at least P90.

All the anatomical features of this system have strong implications for its physiological function and properties which shall be presented in the following section.

I.2 Hippocampal physiology

In this chapter I will briefly review the electrophysiological properties of the hippocampal network in the adult rat and try to emphasise differences to the developing hippocampus. As all the data included this thesis was collected using extracellular in vivo recordings, I will mainly focus on the properties of the hippocampal local field potential (LFP) and single unit activity which can be measured in extracellular recordings. The emphasis will be on the neural mechanisms thought to underlie the generation of oscillations (especially theta oscillations) in HF. I will also briefly discuss synaptic plasticity.

I.2.1 Local field potential

Extracellularly placed recording electrodes can record the local field potential, which reflects voltage changes caused by currents flowing in and out across dendrites over a large group of neurons in an area in some vicinity to the recording site (Buzsáki et al., 2012).

In freely moving rats several types of oscillations over a range of frequencies and correlated to certain types of behaviours can be observed in the LFP of the hippocampal EEG (O'Keefe, 2007): These are delta (1-4 Hz), theta (ca. 4-12 Hz), beta (ca. 10-20 Hz), gamma (ca. 25-140 Hz) and ripple (ca. 140-200 Hz) oscillations. Besides this oscillatory activity, there are also aperiodic multiunit bursts, which give rise to the so-called irregular activity, further subdivided into small irregular activity (SIA) and large irregular activity (LIA) (O'Keefe and Nadel, 1978b; Vanderwolf, 1969). Apart from ripples and delta waves, oscillations in the hippocampal LFP are usually associated with voluntary movement, while irregular activity is linked to quiescence and

consummatory behaviour (Buzsáki, 1986; Buzsáki et al., 1992; O'Keefe and Nadel, 1978b; Vanderwolf, 1969; Whishaw and Vanderwolf, 1973). For reasons of relevance to the project of this thesis I will only briefly review theta oscillations as well as LIA and ripples.

I.2.2 Theta

The most prominent oscillation (i.e. with the highest amplitude) that can be recorded extracellularly in the hippocampus is a relatively slow oscillation in the range of 4-12 Hz, termed theta (Vanderwolf, 1969). The exact frequency of theta depends on a variety of factors including the ongoing behavior (see below), body temperature (Whishaw and Vanderwolf, 1971), running speed (Jeewajee et al., 2008; Sławińska and Kasicki, 1998) and age (Leblanc and Bland, 1979; Wills et al., 2010). Theta oscillations are associated with a variety of behaviours, all of which can be described as voluntary translational movement (e.g. walking, swimming, climbing), and REM sleep (Vanderwolf, 1969; Whishaw and Vanderwolf, 1973). Theta oscillations in the hippocampo-parahippocampal network are most prominent in CA1, but can also be recorded from CA3 and DG (Buzsáki, 2002), as well as from subiculum (Anderson and O'Mara, 2003), entorhinal cortex (Mitchell and Ranck, 1980) and other extrahippocampal brain structures like the amygdala (Paré and Collins, 2000) or cingulate cortex (Leung and Borst, 1987).

Kramis et al. (1975) showed that there are two types of theta oscillations present in the hippocampus: i) type I theta, associated with translational movement (sometimes referred to as t-theta), and ii) type II theta associated with states of arousal, while the animal is more or less immobile (sometimes referred to as a-theta). Since under urethane anesthesia only type II theta is present in the LFP, it could be shown that this type is abolished by the application of

the cholinergic antagonist atropine, while type I theta remains unaffected by the drug in the awake animal (Kramis et al., 1975). Furthermore the frequency of type I theta is higher (7-12 Hz) than type II (4-7 Hz). Bilateral lesions of the entorhinal cortex completely abolish type I theta in the hippocampus, but leave type II theta intact (Bragin et al., 1995; Buzsáki et al., 1983). On the other hand, lesions or pharmacological inactivation of the medial septum completely abolish all theta oscillations in the hippocampus (Lawson and Bland, 1993; Petsche et al., 1962). These results show, that i) type I theta in the hippocampus is entrained by the medial septum via layer II and III cells in EC, and ii) that type II theta is conveyed directly by septo-hippocampal projections.

It is hypothesised (e.g. Buzsáki (2002)) that acetylcholine (ACh) provides a general depolarisation for principal cells and interneurons, while GABAergic projections provide rhythmic inhibitory postsynaptic potentials (IPSPs) to hippocampal interneurons, which are their only targets (Freund and Antal, 1988). Local hippocampal interneurons would then in turn rhythmically inhibit principal cells. This view is further supported by selective toxin-ablation of septal cholinergic cells which only affects theta power but not frequency or occurrence (Lee et al., 1994).

Theta was classically believed to be fairly coherent, i.e. in phase, across the septotemporal extent of each layer in HF (Bullock et al., 1990). Recent studies by Lubenov and Siapas (2009) and Patel et al. (2012) challenge that view, by showing that a theta wave in stratum oriens of CA1 travels like a plane wave from septal to temporal portions of the hippocampus.

I.2.3 Sharp waves

Sharp waves (SPW) are part of the so-called LIA and occur frequently in the hippocampal LFP during periods of immobility, slow wave sleep and consummatory behaviour (Buzsáki et al., 1983; Vanderwolf, 1969), lasting for ca. 50-100 ms (O'Keefe and Nadel, 1978b). They are virtually absent during walking. In the rat pyramidal cells in CA3 and CA1 as well as granule cells in DG show synchronised multiunit bursts during SPW events (Buzsáki et al., 1983). Their electrophysiological basis was deciphered by simultaneously recording the LFP from different depth across the CA1 layer (Buzsáki, 1986; Buzsáki et al., 1983). It is thought that SPWs are triggered by synchronous burst in CA3 during times when extrahippocampal afferent input is absent. SPW events are usually associated with fast oscillations in the LFP (140-200 Hz), so-called ripples (O'Keefe, 1976). Ripples originate in CA1 and there is good evidence that local circuit neurons firing a volley of action potentials at ripple frequency are the source of these oscillations (Buzsáki et al., 1992; Ylinen et al., 1995). Although SPWs and ripples usually co-occur they are indeed separate events as halothane blocks ripples, but not SPWs (Ylinen et al., 1995). SPWs and ripples are believed to play an instrumental role in the strengthening of cell assemblies that were active together, e.g. during exploratory behaviours, based on the fact that during SPWs there is often a replay of sequences of pyramidal cells representing previous experiences (Diba and Buzsáki, 2007; Foster and Wilson, 2006; O'Neill et al., 2008; Skaggs and McNaughton, 1996; Wilson and McNaughton, 1994). This replay is compressed in the time domain. Furthermore disrupting SPWs after a training period impairs spatial learning performance of rats, indicating again the functional significance of these network events for consolidation of memories in the hippocampus (Ego-Stengel and Wilson, 2010; Girardeau et al., 2009; Jadhav et al., 2012).

I.2.4 Single unit activity

In the hippocampus proper, it is possible to distinguish between principal cells and local circuit neurons due to their characteristic firing patterns and action potential waveforms. These are the occurrence of so-called complex spike bursts which typically comprise 2-6 action potentials with decreasing amplitude and with interspike intervals of less than 6 ms (Fox and Ranck, 1975; Harris et al., 2001; Ranck, 1973). This firing pattern is a hallmark of pyramidal cells, confirmed by parallel intra- and extracellular recordings (Henze et al., 2000). In contrast interneurons do not show complex spiking, but exhibit a much more regular firing pattern. Additionally, waveforms of action potentials from interneurons tend to be much narrower (measured from peak to trough) than the ones of principal cells (0.2-0.4 ms vs. 0.4-1 ms) and also their mean firing frequency is usually much higher (30-100 Hz vs. < 1-2 Hz). However, for short periods (< 2 s) pyramidal cells can exhibit a firing rate of up to 20-30 Hz.

I.2.5 Plasticity

The adult hippocampus is the classic mammalian model for studying synaptic plasticity due to the rather easy accessibility of its pre- and postsynaptic neurons in hippocampal slice preparations. In brief, the two most widely studied forms of synaptic plasticity are long-term potentiation (LTP) (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973) and long-term depression (LTD) (Ito and Kano, 1982; Ito et al., 1982). Both mechanisms describe plastic changes at the synapse level, i.e. strengthening or weakening, following high frequency or low frequency stimulation, respectively. LTP formation can be *N*-methyl-D-aspartate (NMDA) receptor-dependent or -independent, both of which occur at different synapses inside the

hippocampus (Collingridge et al., 1983; Harris and Cotman, 1986; Harris et al., 1984). An interesting feature of LTP induction in the hippocampal network is that stimulation with a protocol mimicking several complex spike bursts repeated at theta frequency is particularly effective in inducing LTP in vitro (Larson et al., 1986; Rose and Dunwiddie, 1986). Indeed, if theta is induced in a hippocampal slice preparation by carbachol (cholinergic agonist) application, repeated stimulation at the positive peaks leads to strong synapse specific facilitation, while the same protocol during the troughs does not lead to a strengthening of the postsynaptic response (Huerta and Lisman, 1993). This result has been reproduced in vivo under more physiological conditions in anesthetised animals (Hölscher et al., 1997).

I.2.6 Development of hippocampal physiology

I.2.6.1 Dual role of GABA in early development

Immature neurons and networks can have quite different properties and firing patterns compared to adults. One of the most striking findings was the discovery that GABA, the most abundant inhibitory neurotransmitter in the adult rat brain (Bloom and Iversen, 1971), can depolarise postsynaptic neurons in the developing hippocampus (Ben-Ari et al., 1989). This depolarising response is mediated by $GABA_A$ receptors and is due to an increased intracellular Cl^--concentration of immature neurons (Rivera et al., 1999; Zhang et al., 1991). This effect is thought to enable GABA-mediated responses exerting a dual role: Depolarisation of postsynaptic cells leading to opening of voltage-gated Ca^{2+}-channels (Leinekugel et al., 1995) and activating NMDA receptors (Leinekugel et al., 1997). On the other hand it also shunts the activity of other glutamatergic excitatory inputs on the postsynaptic cell because of its lower reversal potential compared to glutamatergic currents, thus preventing epileptiform activity of the network (Ben-Ari, 2001). This depolarising effect of GABA seems to be a general feature of developing neurons as it has been demonstrated for a variety of structures in the rat brain (Chen et al., 1996; Reichling et al., 1994; Serafini et al., 1995). The switch to the adult $GABA_A$-receptor mediated inhibition occurs postnatally around the beginning of the second week in the hippocampus (Ben-Ari et al., 1989).

I.2.6.2 Development of excitatory synaptic transmission in hippocampus

But it is not only the GABAergic system that undergoes significant maturational changes. It is not until P14 that the adult-like distribution of binding sites of NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in CA1, CA3 and DG is reached, with marked differences between the regions as generally CA3 seems to precede CA1 which in turn precedes DG (Insel et al., 1990). It is noteworthy that there is an overshoot of both receptor binding sites (esp. AMPA receptors in CA3) during the 3rd week, after which it decreases back to adult levels, potentially indicating an elimination of synapses (Insel et al., 1990).

In general the postnatal development of the excitatory synaptic transmission in HCP seems to be more or less fully matured during the 4th week, as stimulation of afferent fibres to CA1 and DG evokes adult-like postsynaptic responses by that time (Bekenstein and Lothman, 1991; Dumas and Foster, 1995). There is good evidence that this is due to a more and more efficient presynaptic transmitter release (Dumas and Foster, 1995).

I.2.6.3 Giant depolarising potentials

The above described depolarising effect of GABA exerts a crucial role in the predominant network activity of the developing hippocampus during the first week of postnatal life (Ben-Ari et al., 1989; Leinekugel et al., 2002). These so-called giant depolarising potentials (GDPs) were first described in vitro in hippocampal slices (Ben-Ari et al., 1989), but correlates have since also been reported in vivo, where they are present as synchronised multiunit bursts (Leinekugel et al., 2002). Both in vitro and in vivo they have a GABAergic and a glutamatergic

component (Ben-Ari et al., 1989; Leinekugel et al., 2002) and represent the main network activity of principal cells and interneurons in HCP until P7 (Leinekugel, 2003).

At P10-12 GDPs are not detectable anymore in hippocampal slices nor is the correlate multiunit activity in vivo in extracellular recordings (Ben-Ari et al., 1989; Leinekugel et al., 2002). Furthermore, the occurrence of GDPs in vivo is strongly correlated with sharp waves which are absent in slice recordings (Leinekugel et al., 2002). The functional significance of GDPs remains to be elucidated, but it is hypothesised that the synchronised activity might provide a basis for a Hebbian type strengthening of immature synaptic contacts in developing networks (Durand et al., 1996; Hebb, 1949; Löwel and Singer, 1992).

I.2.6.4 Sharp waves in development

Behaviourally, multiunit activity in the hippocampus is associated with periods of immobility (Leinekugel et al., 2002). In vivo, SPWs are the predominant oscillatory activity in the hippocampal LFP until P7 (Karlsson et al., 2006; Leinekugel et al., 2002; Mohns et al., 2007). These early SPWs already show a strong similarity in extracellular recordings with the adult type, as they reverse polarity across CA1 with the maximum negative peak in stratum radiatum and their origin in CA3 (Buzsáki et al., 1983; Leinekugel et al., 2002). All these results indicate that GDPs and SPWs most probably represent the same network activity, measured in vitro (GDPs) and in vivo (SPWs), respectively. Subsequently their amplitude increases until P18, while their width decreases in parallel (Mohns et al., 2007). SPWs are initially however devoid of fast ripple oscillations which first occur around P7 (Mohns et al., 2007). The probability of ripple oscillations in SPW events reaches adult levels by ca. P14 (Mohns et al., 2007), although there is a conflicting paper by Buhl and Buzsáki (2005), which reports first ripple occurrence in CA1 only at P14. This difference might be explained by the fact that ripples occur only

infrequently and at low amplitudes until P12-13, which might not have been detected by a spectral analysis used by Buhl and Buzsáki (2005). On the behavioural level, SPWs in neonates are strongly correlated with so-called startles (Karlsson et al., 2006) which are abrupt and simultaneous contractions of skeletal muscles across the whole body (Gramsbergen et al., 1970).

I.2.6.5 Theta in development

During the second week of a rat's life adult-like patterns, i.e. theta and gamma oscillations and ripples, start to dominate the population activity of the developing hippocampus.

The first slow rhythmic oscillations (< 10 Hz) in the hippocampus can be observed around P8-9, although they are quite unreliable in terms of a behavioural correlate and their power in the hippocampal LFP is rather low (Leblanc and Bland, 1979; Mohns and Blumberg, 2008). By P15, a fairly consistent theta of about 5 Hz can be observed in CA1 and DG during voluntary movement, while periods of immobility are accompanied by LIA (Leblanc and Bland, 1979). During the third week theta becomes more strongly correlated with ongoing behaviours, accompanied by a steady increase of frequency and by the 4^{th} week the adult theta frequency of 7-12 Hz is reached (Leblanc and Bland, 1979; Wills et al., 2010). In parallel to the increase in frequency there is also a steady increase in theta amplitude, which reaches adult-like levels during the 4^{th} week (Leblanc and Bland, 1979; Mohns and Blumberg, 2008; Wills et al., 2010). Interestingly there seem to be slight differences in the emergence of theta in HF, as it is observable first in CA3, then in DG and finally in CA1 (Lahtinen et al., 2002; Leblanc and Bland, 1979).

I.2.6.6 Plasticity in development

LTP in CA1 can be robustly induced by a high frequency stimulation around P7-8 with the amount of postsynaptic potentiation steadily increasing until P15, when an absolute maximum is reached, that is even stronger than in adult rats (Harris and Teyler, 1984). This is in line with the observation of an increase in NMDA/AMPA receptor binding sites until the third week in the hippocampus (Insel et al., 1990).

There are also major differences in the mechanisms of synaptic plasticity in the developing hippocampus. LTP formation in the CA1 region of the hippocampus depends on different molecular mechanisms in animals younger than P8 (Yasuda et al., 2003). Additionally, a high frequency tetanic stimulation of the mossy fibres, a protocol known to induce LTP in adults, surprisingly induces LTD in slices from rat pups as young as P11-12, and only results in adult levels of synaptic facilitation around P18 (Battistin and Cherubini, 1994).

I.2.7 Summary of hippocampal physiology

In this section the physiology of the hippocampus was highlighted. Several oscillations (delta, beta, theta and gamma, ripples) can be observed in the hippocampal LFP of adult rats, as well as irregular activity (SPWs). All of these are associated with certain types of behaviours. The origin of theta oscillations was described in more detail, which is meditated to HF by the medial septum and EC. Theta oscillations are believed to arise from the rhythmical discharge of large populations of interneurons. In contrast SPWs and ripples are caused by multiunit activity in CA3 and CA1 respectively, and are present in the hippocampal LFP during periods of immobility. These events are believed to play an instrumental role in the strengthening of previously active connections.

The physiology of the hippocampus of newborn rats is quite different to these patterns, which only start emerging during the second week postnatally. Beforehand the main network activity consists of GDPs which are thought to be important for the strengthening of connections in the immature brain. In parallel the propensity for the induction of synaptic plasticity in the hippocampus develops, which most probably reflects maturation of receptor distributions at the synapse level. In general it seems that by the end of the third/beginning of the fourth week the physiology of the developing hippocampus is more or less similar to that of the adult rat.

I.3 Spatially modulated cells in the hippocampo-parahippocampal network

In this section I will provide an overview of the functional properties of the neurons within the hippocampo-parahippocampal network, whose firing is spatially modulated. While in the last chapter the electrophysiological properties of single units and the population activity of neurons in the hippocampus were described, this chapter will discuss in detail the spatial properties of certain types of identified cell classes, namely place cells, head direction cells and grid cells as well as other spatially modulated cells like boundary vector cells (BVC) and border cells. This is due to the fact that spatial navigation and memory are believed to be the main functions of the rodent hippocampus. For reasons of relevance I will mainly focus on place cells and only briefly discuss the remaining cell types. I will start by reviewing data recorded from adult rats and then go on to discuss what is known about the functional development of these cells.

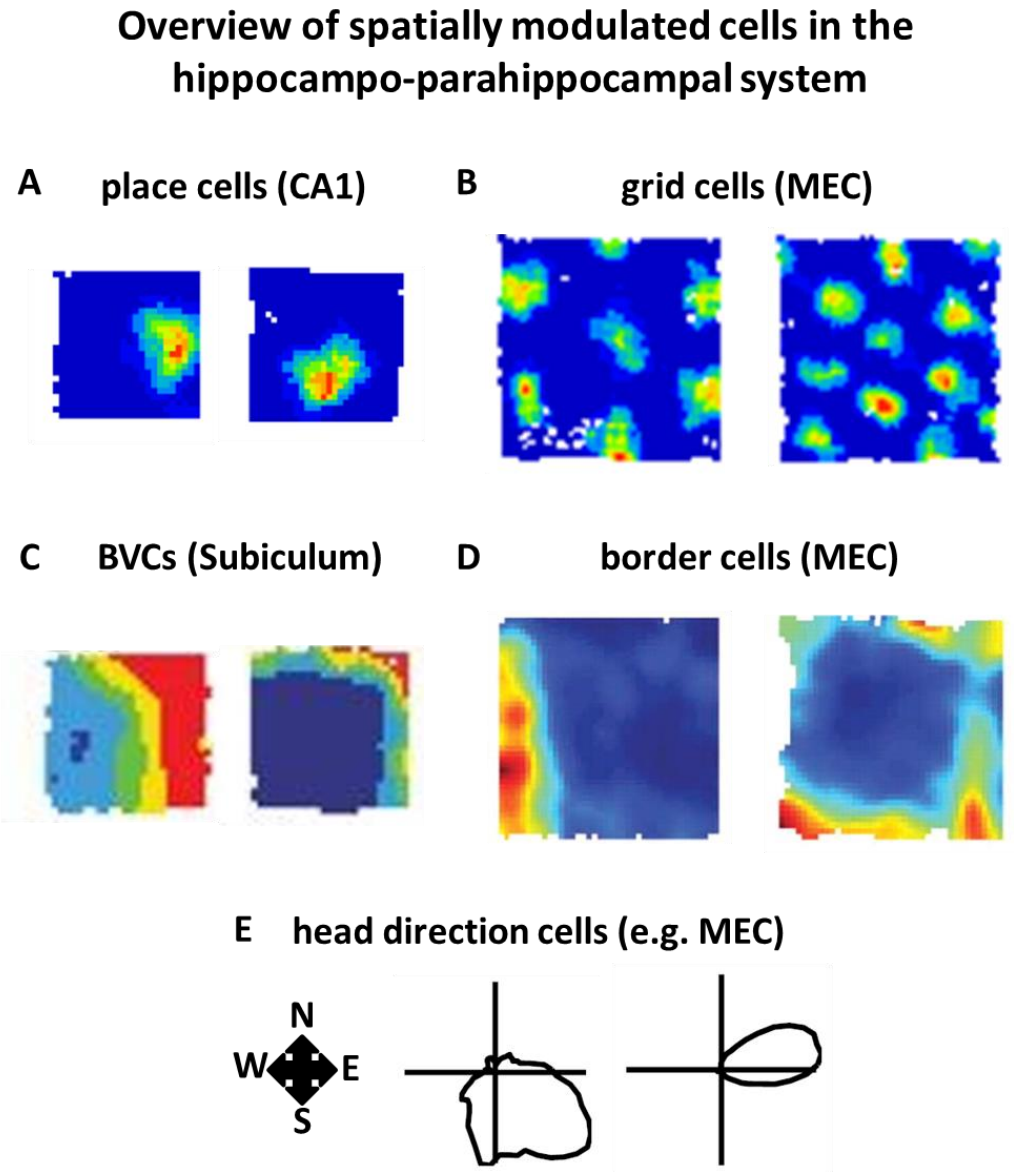


Figure I-6: Overview of firing properties of spatially modulated cells in the hippocampo-parahippocampal network. Firing rate is shown as function of location in A-D. Progressively warmer colours indicate progressively higher firing rates. Size of rat maps indicates relative environment proportions. A: two examples of place cells recorded from CA1. B: two examples of grid cells recorded from MEC (courtesy of T. Wills). C: two examples of boundary vector cells (BVCs) recorded from subiculum (adapted from Lever et al. (2009)). D: two examples of border cells recorded from MEC (adapted from Solstad et al. (2008)). E: two examples of head direction cells recorded from MEC (courtesy of T. Wills). Firing rate is presented as function of heading direction in polar coordinates (see compass inset).

I.3.1 Place cells

Ever since their discovery in the 1970's (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976) place cells have attracted the interest of researchers all around the world due to their relative simple identification in extracellular in vivo recordings in freely moving rodents. When O'Keefe and Dostrovsky (1971) placed recording electrodes in the CA1 area of freely moving rats, they noticed that the firing of individual units correlated best with a certain specific location in the recording environment, instead of any kind of the ongoing behaviour. Individual cells fired volleys of action potentials when the animal visited certain parts of the testing environment while being more or less silent in all other parts. Because each cells was most active at a unique location they named these cells 'place cells' and the area in the environment of its maximum activity its 'place field' (O'Keefe, 1976) (see Figure I-6A).

Place cells in the hippocampal formation are found predominantly in the CA1 (O'Keefe and Dostrovsky, 1971) and CA3 (Olton et al., 1978) subfields of the hippocampus proper, but have been described in other areas, like e.g. subiculum (Sharp and Green, 1994) and EC (Frank et al., 2000; Quirk et al., 1992). Since by far the most data in the literature is obtained from recordings from septal portions of CA1 and CA3 I will mainly focus on the properties of place cells found in these regions.

I.3.1.1 Anatomical identity of place cells

Anatomically there is good evidence that place cells correspond to pyramidal cells in CA1 and CA3 (Henze et al., 2000). Interestingly, there seems to be no topography of place cells in the hippocampus, in that there is no recognisable correlation between anatomical location and

place field proximity in a testing environment. Neighbouring cells in the hippocampus (i.e. recorded from the same electrode) do not display place fields located in close proximity in experimental environments, compared to cells recorded from different electrodes (Redish et al., 2001).

I.3.1.2 Basic properties of place cells

Firing rates of place cells

As a rat traverses a place field of a given place cell in a standard laboratory open field enclosure, the cell, on average, starts to fire increasing numbers of action potentials until the centre of the field is reached, after which the firing rate decreases again (Muller et al., 1987). In the field centre average firing rates of up to 20-30 Hz can be reached, while the firing frequency outside the field usually approximates 0 Hz (Muller et al., 1987).

Apart from this firing rate code for signaling the animal's position, there is another coding mechanism embedded in place cell firing. The position of the animal is not thought to be encoded only through a rate code, but also through a temporal code. O'Keefe and Recce (1993) were the first to notice an intriguing correlation between place cells firing and the ongoing theta rhythm, when they recorded place cells from rats running along a linear track. As a rat entered a place field, the place cell emitted spikes at the trough of the theta oscillation in the simultaneously recorded LFP (Skaggs et al., 1996). As the animal traversed the place field, spikes were emitted at progressively earlier phases of the ongoing theta oscillation. This phenomenon was called 'phase precession'. O'Keefe and Recce (1993) could show that the

phase of firing correlated best with the animal's position inside the place field and not with e.g. time since entering the field or other behavioural parameters.

Directionality of place cells

Place cell recordings in open field enclosures showed that firing rates of place cells are not strongly modulated by the direction of the animal's movement through a place field (Muller et al., 1987; O'Keefe, 1976). Surprisingly, when place cells are recorded on linear tracks or narrow multi-arm mazes they show a strong directional modulation (Huxter et al., 2003; McNaughton et al., 1983; O'Keefe and Recce, 1993). That is, they are only active during runs in one direction while being silent during runs in the opposite direction. These are not distinct populations of pyramidal cells, but the same cells are non-directional in the open field and become directional on a radial arm maze or when animals repeatedly run between specified goal locations in an open field (Markus et al., 1995; Muller et al., 1994). A recent study by Navratilova et al. (2012) demonstrated that directionality of place cells on linear tracks is not an inherent property of these cells, but develops with experience on the track. Initially place cells are also bidirectional on linear tracks.

I.3.1.3 Place field properties

Shape of place fields

The shape of place fields in open field enclosures is usually circular or elliptical for fields in the environment centre, irrespective of the shape of the testing environment. Place fields are

crescent or half-circle shaped when situated along walls depending on whether the recording environment is comprised of a circular or a rectangular enclosure (Muller and Kubie, 1987; Muller et al., 1987).

Size of place fields

The sizes of place fields of septally recorded units from CA1 can differ to some extent when recorded in standard laboratory environments (Brun et al., 2002; Muller et al., 1987). These are the only fairly recent studies to my knowledge that present a quantitative assessment of place field size across the whole population that was recorded. In both studies place field size ranges between ca. 5-60% of the recording environment with a global average field size of ca. 10-20% of the testing environment. This is in line with other studies which only report the average field size and translates to roughly 400-700 cm^2, independent of whether the recording environment was an open field enclosure or a linear track (Brun et al., 2002; Henriksen et al., 2010; Jung et al., 1994; Maurer et al., 2005; O'Keefe and Recce, 1993; Park et al., 2011; Poucet et al., 1994). Differences between individual studies might at least in part be due to the fact, that field size generally seems to increase with the size of the environment (Fenton et al., 2008; Henriksen et al., 2010; Kjelstrup et al., 2008; Muller and Kubie, 1987; Park et al., 2011). The increase seems to be much stronger for CA1 compared to CA3 (Park et al., 2011). This rescaling of place field size is a general feature of place cells as it is apparent in open field enclosures and on linear tracks.

Place field size also increases from septal to temporal CA1 in a somewhat graded fashion, such that cells recorded more temporally have larger firing fields. Near the temporal poles place fields are on average twice as large as the ones recorded from septal poles (Jung et al., 1994; Maurer et al., 2005). This increase in spatial scale of place cells at more temporal recording

locations has also been reported for CA3 place cells on a linear track as well as in open field enclosures (Kjelstrup et al., 2008).

One general caveat of comparing results on place field properties like size or numbers of firing fields across studies is that there is no consensus of how a firing field is defined. Researchers employ different criteria for e.g. minimum size and/or minimum firing rate to define the field border. This is further complicated by the fact that different laboratories also use different methods for the construction of rate maps (i.e. different smoothing algorithms).

Numbers of place fields

In standard laboratory open fields (< 1 m^2) most place cells in the more septal regions of CA1 only have one firing field (Muller and Kubie, 1987; Muller et al., 1987). Different estimates exist about the percentage of place cells which have more than one firing field. In Muller et al. (1987) and Jung et. al (1994) these comprise ca. 20% of the recorded population, while in a study by Fenton et al. (2008) this proportion is 28%. In Henriksen et al. (2010) the average number of fields per cell is reported to be around 1.1 which would translate to roughly 10% of the recorded place cell population having more than one field.

However, the proportion of cells with more than one field increases dramatically, if place cells are recorded in larger environments (> 2 m^2). Here the majority (ca. 80%) of CA1 and CA3 place cells show multiple place fields (3-5 fields on average) when recorded in a standard large box as well as in a more complex environment with some three-dimensional aspects (Fenton et al., 2008; Park et al., 2011). Interestingly, there seems to be no systematic relationship between the locations of multiple firing fields in an individual cell, unlike in entorhinal grid cells (Hafting et al., 2005). Interestingly, for linear tracks the correlation between field number and

environment size does not seem to exist as CA3 place cells do not show an increase in numbers of firing fields when recorded on an 18m-long linear track (Kjelstrup et al., 2008). It is noteworthy that Kjelstrup et al. (2008) also recorded place cells from CA3 in a large circular enclosure (diameter (Ø): 2 m) but surprisingly do not explicitly report an increase in field number per cell.

Additionally, there is an interesting relation between the anatomical location of a place cell along the transverse axis of CA1 and its average number of fields per cell (Henriksen et al., 2010). Cells located more proximally (i.e. near the border to CA3/CA2) tend to have rather fewer place fields in a large circular enclosure (Ø 2 m), than cells recorded from more distal regions of CA1 (i.e. near the border to SUB). Henriksen et al. (2010) report a gradient-like increase in the number of place cells with more than one place field (ca. 40% for proximal and intermediate regions and ca. 70% for distal regions) and also an increase in average number of fields per cell (ca. 1.6 for proximal and intermediate regions and ca. 2.1 for distal regions). This gradient was also reported for a standard size environment (Ø 1 m), but with a much smaller change between different proximodistal positions of CA1.

Distribution of place fields in testing environments

Muller et al. (1987) report a uniform distribution of place fields across the environment in an open field enclosure, while another study (Hetherington and Shapiro, 1997) reports specific clustering of place fields near the walls, especially those with polarising (i.e. salient) cues. There is also one report of place field clustering around the escape platform in a water maze task (Hollup et al., 2001).

On linear tracks there is sometimes a skewed distribution of firing fields towards the end points of the track which can be overrepresented in comparison to more central parts (Dombeck et al., 2010; Ziv et al., 2013). However, in a different study Gothard et al. (1996) report a uniform distribution of place fields along a linear track.

I.3.1.4 Time course for establishment of place fields

Wilson and McNaughton (1993) report that stable and reliable fields in a previously unvisited part of a recording environment can be observed after ca. 10 min, while Frank et al. (2004) show in a more detailed analysis that on a T-maze stable location specific firing can be observed as fast as 2 min. Furthermore place cells usually keep their firing fields in a given environment over the course of repeated recording trials, and can actually be stable for at least a week (Lever et al., 2002). There even is a report of place field stability for months (Thompson and Best, 1990), but these cells were recorded with single electrodes, and it is thus hard to know for sure whether really the same units could be followed over such a long period.

I.3.1.5 Ensemble firing of place cells

Active vs. silent cells

If the same place cell population is recorded in sufficiently different environments (i.e. environments in different experimental rooms or e.g. environments with different features like shape, wall and/or floor texture and/or colour), and the animal had a fair amount of experience in these, it becomes clear that a substantial fraction of the place cell population is

not active in one (or more) of the chosen environments (Bostock et al., 1991; Muller and Kubie, 1987; O'Keefe and Conway, 1978; Thompson and Best, 1989). The active cells, referred to as the active subset, thus only represent a certain fraction of all place cells that are located in the vicinity of the tips of the recording electrodes and could potentially be recorded. The question therefore is how big is this fraction on average? Thompson and Best (1989) recorded place cells in three different environments and under light anesthesia (pentobarbital) with the latter condition known to increase spontaneous firing in pyramidal cells (Ranck, 1973). They concluded that only about 35% of all complex spike cells identified under anesthesia have a place field in at least one of the environments tested. Other studies classified 30-45% (Wilson and McNaughton, 1993) and 70% (Gothard et al., 1996) of the total population of recorded complex spike cells as place cells. These percentages are in line with a study assessing the expression of the immediate-early gene *Arc* (Lyford et al., 1995) in CA1 and CA3 after animals explored two different environments (Guzowski et al., 1999). All these results show that the code for the representation of space in the hippocampus is relatively sparse, a mechanism to potentially maximise the number of possible unique environment representations.

Remapping of place cells

The term 'remapping' of place cells broadly describes the effect of the behaviour of different active subsets of place cells in different environments. It not only refers to the switching on and off of place cells in different environments, but also to the shift of place fields to an unpredictable new location between different environments (Bostock et al., 1991; Muller and Kubie, 1987; O'Keefe and Conway, 1978). The termed was originally coined by Bostock et al. (1991) who differentiated between 'rotational' (i.e. shift of the firing field to a location that can be achieved by a simple rotation of the original firing field) and 'complex' (i.e. shift of the

field to a new unpredictable location) remapping. Throughout the literature various terms for slightly different types of remapping are used. Some researchers refer e.g. to 'partial' (Knierim, 2002; Moita et al., 2004; Skaggs and McNaughton, 1998) and 'global' (Leutgeb et al., 2005b) remapping, depending on whether remapping occurs only in a subset or the whole population of the recorded cells. Others use the term 'rate remapping' for the special case, when place field locations remain unchanged between two environments, but robust differences in average firing rates across both environments exist (Leutgeb et al., 2006, 2005b). In this thesis remapping will generally refer to global changes in place cell firing, including both the shift of a firing field to a new location as well as the environment specific activity/ceasing of activity of place cells. The exact factors and the neuronal basis for remapping are still not fully understood. However, a few of the determinants that drive remapping of place fields have been elucidated and several theories about their neuronal basis do exist.

In their original study Bostock et al. (1991) trained rats on a random foraging task in a grey cylinder with a white cue card. Then the animals were probed in the same environment with either the white or a novel black card. When comparing the individual firing fields of place cells recorded in both cylinder configurations they noticed that after less than two trials with the black card present field locations were rather similar to the white card configuration (place cells had homotopic fields or showed rotational remapping). Only a smaller subset showed complex remapping. However, this ratio changed dramatically after more than two exposures to the black card configuration, as now a much larger proportion of place cells (ca. 75%) showed complex remapping between both card configurations. Moreover, the exact time point of the shift between rotational and complex remapping also varied between individual animals and seemed to occur rather sudden.

This was confirmed in a study by Lever et al. (2002) who recorded place cells over the course of several weeks in a square and circular open field. Initially the location of the firing fields of

individual cells is very similar in both environments but over time the spatial representation became more and more dissimilar. Lever et al. (2002) also report major differences between individual animals for the time course of this shift. Additionally, they also show that even cells from an individual animal vary in the time point when exactly remapping occurs.

Leutgeb and colleagues put forward the functional difference between rate and global remapping (Leutgeb et al., 2005a, 2006, 2004; Leutgeb and Leutgeb, 2007). They argue that global remapping occurs e.g. when there is a change in spatial location (i.e. recordings in different rooms) and rate remapping occurs when the location (with respect to the recording room) remains constant but the cue context changes (i.e. e.g. recording in environments of different colours or shapes in the same physical location). They furthermore emphasise that the ensemble of place cells in CA3 and CA1 show marked differences between the conditions when these two forms of remapping occur (Leutgeb et al., 2005b, 2004). Place cells in CA1 show less pronounced rate remapping and react more strongly to a change in the local cue context. Recordings in similar environments in different rooms only lead to a partial change of the spatial representation in CA1. In contrast place cells in CA3 show strong rate remapping when recorded in different environments in the same physical location, and a much more pronounced orthogonalisation between environments in different recording rooms. The authors argue that these differences point out to different types of 'spatial maps' in both CA subfields (Leutgeb and Leutgeb, 2007). In CA1 the spatial representation is stronger bound to the sensory cue context and can have different maps for the same physical location, while in CA3 a unique map is formed for a given location in space which could contain additional information conveyed by changes in firing rates.

While these differences between CA3 and CA1 place cells and the idea of two forms of remapping signaling different environmental changes can explain some results (e.g. similar locational firing patterns in a square and a circle in the same recording position) it fails to

explain e.g. why in Bostock et al. (1991) a change of the cue card colour induces global as well as rate remapping. In similar experiments in the Moser laboratory nearly all cells in CA3 and CA1 showed only rate remapping (Leutgeb et al., 2005b).

Finally, I want to stress that in contrast to this rather homogenous behavior of the whole recorded cell population to environmental manipulations, other experiments (e.g. incongruent rotation of local and distal cues (Renaudineau et al., 2007; Shapiro et al., 1997)) show that individual place cells in one animal can indeed react with marked differences (i.e. remap or follow one set of cues). All these results indicate that remapping is not a fixed process underlying general rules, but critically seems to depend on various factors like previous experience of the animals (Leutgeb et al., 2005a; Wills et al., 2005), task demand (Markus et al., 1995) and inter-animal variability (Bostock et al., 1991; Lever et al., 2002).

Pattern separation and pattern completion

In broad theoretical terms remapping is inevitably linked to two general hypothesised processes of neuronal computing, pattern separation and pattern completion (Hunsaker and Kesner, 2013; Leutgeb and Leutgeb, 2007; Marr, 1971; Rolls, 1996). The former refers to the orthogonalisation of spatial representations in the face of similar inputs, like the progressive difference between place field locations of individual cells in different shaped environments in the same recording position (e.g. Lever et al. (2002)). The latter process describes the opposite effect, namely the preservation of spatial representations, if only part of the original input is present. An example for this would be the persistence of place cell firing in an environment in the absence of some cues used in the original configuration (Fenton et al., 2000; O'Keefe and Conway, 1978).

The standard hypothesis is that pattern separation is a main function of DG, based on synaptic plasticity at the perforant path-to-granule cell synapses and the connectivity pattern of granule cells to CA3 which is rather sparse, whilst pattern completion is proposed to occur in CA3, based on the extensive recurrent collaterals of CA3 pyramidal cells (for reviews on this matter see Rolls and Kesner (2006) or Hunsaker and Kesner (2013)). However, the experimental evidence supporting this hypothesis is limited to a handful of studies. In one elegant study, Nakazawa and colleagues (Nakazawa et al., 2002) could convincingly show that by selective deletion of NMDA receptors in CA3 pyramidal cells, a specific deficit resembling an impairment in pattern completion could be induced in mice. Wills et al. (2005) showed that, if place cells which were remapped between two shapes (square and circle) were then presented with a set of intermediate shapes, the entire place cell population appeared to make a coherent, all-or-none categorisation of these shapes as either square or circle: no intermediate firing patterns were seen. This result is suggestive of the hippocampus acting as an attractor network (Marr, 1971), as well as being consistent with both pattern separation and pattern completion in the hippocampus, but see Leutgeb et al. (2005a).

I.3.1.6 Determinants of place cell firing

Geometry of environmental boundaries

The first evidence that the distance to certain environmental boundaries might be an important factor for place cell firing came from an experiment by Muller and Kubie (1987) in which they recorded place cells in two visually identical cylindrical or two visually identical rectangular enclosures that only differed in their size. Most of the place cells that were active in both environments of identical shape (ca. 50% of all recorded cells) had place fields in the

same angular position in each enclosure but scaled up in size when recorded in the larger version. It is noteworthy that the scaling was not perfect since the large environment represented a fourfold increase in size, while place field sizes on average only increased two-fold.

In a seminal study O'Keefe and Burgess (1996) showed that by stretching one or two sets of walls of a small square to a rectangle or a large square respectively, place fields stretched proportionally in the direction of the environmental expansion, develop a double peak or cease firing. Their results showed that peak firing positions of nearly all place cells in CA1 is determined by a certain distance to one or more walls of the environment and/or the aspect ratio of the distance between two walls (a very small subset was rather fixed to the recording room walls). Additional evidence for the influence of environmental boundaries on place field position comes from a study conducted by Lever et al. (2002) showing that small shifts of the same box does not influence place field position, while removal of environmental walls leads to remapping of the majority of place cells.

Sensory control of place cell firing

Sensory control of place cell firing describes the fact that place fields in an environment can be anchored to certain sensory cues/stimuli. These can be external (to the animal) cues in the environment (landmarks, features of environment, etc.) or internal ones caused by the movement of the animal like e.g. information about self-motion (path integration). External cues can of course be of different sensory modalities, e.g. tactile, visual, auditory or olfactory cues. In general one has to be cautious when trying to understand which types of cues are the dominant ones for place cell anchoring, since often certain sets of sensory cues will

intentionally be made unreliable by the experimenter, e.g. by cleaning the floor of an environment in between recording trials.

One approach used to understand the importance of the presence of certain sensory cues over place cell firing has been that of eliminating (e.g. through lesion) specific sensory inputs. For instance, what happens in the total absence of visual information? Save et al. (1998) recorded place cells from rats blinded shortly after birth by surgical removal of the eye balls (at P7). They found that place cells recorded from blind rats (once the animals reached adulthood) had very similar properties to the ones from sighted rats. The only observed differences to place cells recorded from sighted rats were a lower firing rate and a stronger reliance on tactile cues inside an environment. However, blinding rats very early in development might lead to a compensation by other sensory modalities, and thus only shows that place cell firing is generally not altered by a permanent absence of visual input. Hill and Best (1981) in an earlier study had deafened rats pharmacologically and blindfolded them, before recording place cells in a radial arm maze. Their results showed that place cell firing appears normal under such conditions, but is now tightly bound to local cues inside the maze. This shows that visual input is not necessary for normal place cell function in sighted rats and that place cells can compensate the absence of visual information by using cues of other modalities (tactile and olfactory) that signal a certain location in the testing environment.

Other studies did record place cells in total darkness in untreated animals in open field enclosures as well as in a radial arm maze (Markus et al., 1994; Quirk et al., 1990; Save et al., 2000). The general result is that place cell firing and place field properties are more or less similar to the ones recorded under light conditions, with a trend to convey less spatial information and to be less coherent in darkness. Interestingly, if the animal is inside the environment when the lights are extinguished, place fields usually keep their fields from the light condition (Quirk et al., 1990; Save et al., 2000), while the majority (ca. 50-60%) remap

when the animal is placed in the environment in the dark (Markus et al., 1994; Quirk et al., 1990). These results demonstrate that if visual information is removed after the animals had already explored the environment in the light, place cells can compensate this by either using the remaining sensory cues inside the environment or use information about their self-motion to update the internal estimate of the current position. The remapping of place cells when animals are put into the environment in the dark highlights the general importance of visual cues for place cell firing.

In a study by Save et al. (2000), the authors tried to investigate the importance of olfactory cues in light and dark conditions by either cleaning or not cleaning the floor of the testing apparatus, while the animal was inside the environment (this resulted in four conditions: light/cleaning, light/no cleaning, dark/cleaning and dark/no cleaning). Scrambling the olfactory cues in combination with an absence of visual information had a profound effect and led to remapping of nearly all cells, although the animal was inside the environment during the whole procedure. Moreover, it showed that when the same protocol was done in light conditions the amount of remapping was nearly equally strong, while most fields remained stable when the floor of the environment was not cleaned. This shows that olfactory cues can exert some control over the location specific firing of place cells, albeit a weaker one than prominent visual cues, at least over the course of a recording session during which the animal is not removed from the environment. The fact that local tactile and/or olfactory cues appear to provide much weaker control over place cell firing than visual cues can probably be explained by the fact that usually experimenters are deliberately trying to minimise their influence (by cleaning the environment in between trials or exchange certain parts of an environment with visually identical copies). These manipulations might result in the animals ignoring these unreliable stimuli for signaling a certain location.

Control of individual cues over place cell firing

The above described studies all contained experiments where all or at least most stimuli of a certain modality were manipulated. These results therefore do not allow any assumptions about the specific control of individual stimuli over place cell firing.

The first evidence that individual cues in an environment can indeed exert control over place cell firing was gathered by recording place cells in a cue-controlled environment and rotating the environment in parallel to the cues (O'Keefe and Conway, 1978). If the maze was rotated in unison with the cues (keeping their spatial relationship intact), place fields of most recorded units rotated correspondingly. Furthermore, this work showed that most place fields were anchored by a configuration of at least two cues, as removal of any two cues left nearly all place fields intact, while the removal of more than two cues led to strong changes in the locational firing of place cells. The exact cues which seemed to exert the strongest influence on a given unit varied from cell to cell, although visual ones seemed to generally dominate. This shows the hippocampus ability to re-activate spatial representations even in situations when only a subset of the original cue configuration is present. Only once the mismatch exceeds a certain threshold a new representation is formed by place cells.

The effect of cue control by polarising sensory (esp. visual) cues has been reproduced in many subsequent studies with a larger cell population and better positional sampling of the environment (Bostock et al., 1991; Fenton et al., 2000; Hetherington and Shapiro, 1997; Muller and Kubie, 1987; O'Keefe and Speakman, 1987). All these studies show that a rotation of the polarising cue ('cue card') in curtained environments is accompanied by a corresponding rotation of place field locations. This demonstrates that place cells are able to learn about the exact spatial relationship between a landmark ('visual cue') and certain positions inside the environment. However, this effect depends also on the animals experience with the stability of

the visual landmark, as they ignore rotations of the cue card, if e.g. they saw it being moved by the experimenter (Jeffery and O'Keefe, 1999; Jeffery, 1998; Jeffery et al., 1997; Knierim et al., 1995; Rotenberg and Muller, 1997) which demonstrates the hippocampus ability to use only information about the actual position of the animal which is 'judged' reliable.

The overall picture of cue control of place cell firing is further elucidated by experiments where the recording apparatus (proximal cues) and the distal cues (outside recording apparatus) are rotated in opposite directions or one of the cue configuration is scrambled (Renaudineau et al., 2007; Shapiro et al., 1997; Tanila et al., 1997a). Results from such experiments indicate that the majority of place cells (ca. 50%) are anchored to the specific configuration between both cue sets, since these cells remap when a large mismatch between proximal and distal cues is created. The exact influence of individual cues in a specific configuration is nonetheless still an open question. While some researchers report no effect of the removal of individual or all of the distal salient cues in a familiar environment (Fenton et al., 2000; O'Keefe and Conway, 1978; O'Keefe and Speakman, 1987) others do report slight changes in the firing properties of individual cells after a removal of individual cues (Hetherington and Shapiro, 1997).

I.3.1.7 Differences between place cells in CA3 and CA1

At the single cell level firing properties between place cells recorded from CA3 and CA1 are very similar (Barnes et al., 1990; Leutgeb et al., 2004; Muller et al., 1987; Renaudineau et al., 2007). In both regions principal pyramidal cells show complex spiking and the formation of place fields. Some different reports exist about either a higher spatial specificity in CA1 place cells (McNaughton et al., 1983) or those recorded from CA3 (Barnes et al., 1990; Park et al., 2011). Nonetheless, place cells recorded from both subfields are often pooled for the respective data analysis in various studies across the literature (Bostock et al., 1991;

Renaudineau et al., 2007; Save et al., 2000; Siegel et al., 2008; Tanila et al., 1997b; Wilson et al., 2004).

But, as already mentioned in the section about remapping (see section 'Remapping of place cells', p. 63) there are some stronger differences in terms of the behavior of the whole cell ensemble (Lee et al., 2004; Leutgeb et al., 2005b, 2004; Vazdarjanova and Guzowski, 2004), especially which environmental changes lead to which form of remapping (rate vs. global). An exact description of these differences is beyond the scope of this thesis, but the reader is referred to a comprehensive review on this matter by Leutgeb and Leutgeb (2007).

I.3.2 Other spatially modulated cells in the hippocampo-parahippocampal network

I.3.2.1 Head direction cells

Head direction cells (HD cells) are cells which start discharging when an animal's head is facing into a certain direction (Taube et al., 1990a) (see Figure I-6E). This so-called preferred firing direction is different for each cell and all possible directions are equally distributed in the cell population (Taube et al., 1990a). HD cells were originally described in the postsubiculum (Taube et al., 1990a, 1990b), but have subsequently been found in a large variety of brain structures. This includes different parts of the classic Papez circuit (Papez, 1937) like the anterior dorsal thalamic nucleus (ADN) (Taube, 1995), lateral mammillary nuclei (Stackman and Taube, 1998), retrosplenial cortex (Chen et al., 1994) and EC (Sargolini et al., 2006) as well as

other brain areas like lateral dorsal thalamus (Mizumori and Williams, 1993) or striatum (Wiener, 1993) (for a review see Taube (2007)).

Functionally head direction cells are usually characterised by their directional tuning curves. These curves typically have a Gaussian shape and show that this cell type increases its firing rate approximately linear once the preferred firing direction is approached by the animal's head, while the rate is approximately zero when the animal is not facing in that direction.

One important feature of HD cells is that they are not anchored to the earth's magnetic field, but to a specific allocentric direction defined by one or several salient landmark(s) (Taube et al., 1990b). Hence, HD cells will have different preferred firing directions in two distinct environments. Furthermore, HD cells behave in a very similar fashion to place cells upon rotations of salient visual cues (i.e. e.g. a cue card). Just like place fields, the preferred firing directions of HD cells rotate with approximately equal amounts as the cue (Taube et al., 1990b).

There is good evidence that the rotation of place fields is under the control of head direction cells. First, place fields and simultaneously recorded HD cells tend to rotate in unison by similar amounts, when animals were disoriented (Knierim et al., 1995). And second, lesions of PoS result in poor cue control of HD cells in the ADN as well as in place cells, although generally leaving the formation of place fields largely intact (Calton et al., 2003; Goodridge and Taube, 1997).

I.3.2.2 Grid cells

Since the major input to the hippocampus originates in EC, it was long hypothesised that a spatially modulated signal should also be present in this parahippocampal region. Quirk et al.

(1992) reported cells with location specific firing properties in the superficial layers of MEC that were vaguely resembling place cell firing, albeit with a weaker spatial tuning and differences in their response to environmental manipulations.

A more systematic choice in their recording locations lead to the discovery of a novel type of spatially modulated cells in the caudal portions of MEC in the Moser laboratory more than ten years later (Fyhn et al., 2004; Hafting et al., 2005). Due to their intriguing spatially modulated firing patterns these cells were termed 'grid cells' (Hafting et al., 2005). In contrast to place cells which typically only have one firing field in normal laboratory environments, the firing fields of these cells tessellate an open field enclosure in repeated approximately equilateral triangles, forming a highly regular pattern (Hafting et al., 2005) (see Figure I-6B). Anatomically grid cells are thought to correspond to principal cells in MEC, that is stellate and pyramidal cells (Domnisoru et al., 2013; Schmidt-Hieber and Häusser, 2013).

Originally grid cells were identified in layer II of MEC (Hafting et al., 2005), but they have now been found in all principal cell layers of MEC (Sargolini et al., 2006), as wells as in PrS and PaS (Boccara et al., 2010). Grid cells in layer II are typically omnidirectional, while in deeper layers most grid cells are directionally modulated (conjunctive cells) (Sargolini et al., 2006).

The anatomical location in the dorsomedial-ventrolateral axis of MEC determines certain properties of grid cell firing. With increasing distance from the postrhinal border the spacing and sizes of individual grid peaks increases (Barry et al., 2007; Brun et al., 2008b; Hafting et al., 2005; Sargolini et al., 2006), just as place cells recorded from more ventral parts of the hippocampus have larger firing fields than those from dorsal portions (Jung et al., 1994).

Due to their regularly spaced firing peaks, grid cells were immediately thought to possibly provide the navigational system with a metric signal (Moser and Moser, 2008). However, grid cell firing seems not to be an absolute metric signal, at least not at all times, as recently it

could be shown that grid cells show a novelty-induced expansion of their scale and firing peaks (Barry et al., 2012a).

Finally there are some interesting functional relations between grid cells and place cells. Global place cell remapping is associated with realignment of grid orientations (Barry et al., 2012a; Fyhn et al., 2007), while rate remapping in place cells is associated with stable grid fields (Fyhn et al., 2007). Moreover, some computational models predict the location specific firing of place cells to arise from the combined input from several grid cells (Blair et al., 2008; Solstad et al., 2006). However, when direct input from grid cells to CA1 is abolished by lesions, location specific firing of place cells in CA1 persists, albeit a reduction in the quality of the spatial tuning is present (Brun et al., 2008a). Interestingly, if the hippocampus is inactivated by an injection of muscimol, the spatial tuning of grid cells degrades completely and surprisingly grid cells acquire a directional tuning in parallel (Bonnevie et al., 2013).

I.3.2.3 Boundary vector cells and border cells

Boundary vector cells (BVC) are spatially modulated cells found in the dorsal subiculum, that fire at a certain distance to environmental boundaries at a certain allocentric direction (Lever et al., 2009) (see Figure I-6C). A boundary can consist of a wall or large obstacle as well as of a drop on the edges of an open platform that is located above the floor. Different BVCs are tuned to different distances from environmental boundaries with a bias for shorter distances (Lever et al., 2009). One main difference to place cells is that BVCs do not remap in different environments, but that they are solely tuned by the above mentioned parameters, irrespective of shape or other cue contexts of different environments. Their firing properties (rate and waveform) indicate that they likely correspond to principal pyramidal cells in the subiculum (Lever et al., 2009).

BVC are a classic example of a theoretical construct that has been hypothesised before such cells had actually be recorded from HF in rats (Barry et al., 2006; Hartley et al., 2000). Although the model which describes place cell firing by a thresholded linearly summed input from several different BVCs is rather simple, it can quite accurately describe the change in locational specific firing of place cells in environments of different geometry.

Border cells in all layers of MEC were identified and described in parallel to the discovery of BVCs (Solstad et al., 2008). These cells have some similarity to BVCs, but also show some marked differences. Border cells tend to fire along one or several walls of an environment, which is one of the differences to BVCs, as some units fire along all four walls of a square environment (see Figure I-6D). Furthermore only a very small subset seems to be tuned to fire at a certain distance to environmental boundaries. Most border cells have firing fields just adjacent to the wall(s). Another marked difference is that border cells tend to remap when all walls are removed and the border of the environment now is constituted by a drop. It is so far not clear whether border cell firing is based on inputs from BVCs, or if these two cell types are independent of each other.

I.3.3 Functional development of spatially modulated cells

Only four studies have so far recorded place cells and/or head direction cells and grid cells in very young animals (< P50), in an attempt to assess their properties and functional development (Langston et al., 2010; Martin and Berthoz, 2002; Scott et al., 2011; Wills et al., 2010). All studies recorded place cells in young rats, while head direction cells were recorded in three (Langston et al., 2010; Martin and Berthoz, 2002; Wills et al., 2010) and grid cells in two (Langston et al., 2010; Wills et al., 2010) of the studies. In general, I will focus on the results of the three most recent papers, as the earliest study from Martin and Berthoz (2002) only contains a very small sample of units (27 complex spike cells; 3 HD cells) in comparison to the others. The reason for this generally rather scant amount of data on this topic is due to the technical difficulties of conducting these kinds of experiments (esp. chronic implantation of recording electrodes) with young rats.

I.3.3.1 Place cells in development

Both Wills et al. (2010) and Langston et al. (2010) report an increase in the proportion of place cells in the CA1 region from their first occurrence at P16 and P28 or P35, respectively.

This is accompanied by a steady increase in place field stability both across and within recording trials. For both types of correlations adult levels are reached around P31 (Langston et al., 2010). Wills et al. (2010) report a significant increase in the quality of the locational firing of place cells between P16 and P28 (measured by spatial information content). Spatial information (Skaggs et al., 1993) measures the information content of individual spikes and is an indicator of how well the animals' position can be predicted on the basis of the spikes fired

by an individual cell. While in this study adult levels of average spatial information content per cell are not reached at P28, Langston et al. (2010) report similar average values to adults as early as P16, although there is a non-significant trend for a small increase between P16 and P35. This is surprising since both studies use the same method for place cell definition, the same smoothing algorithm for constructing firing rate maps and the same recording system.

What is even more surprising is the fact that in the Scott et al. (2011) study a very similar functional development with increasing age is reported, albeit starting at a later time point (P23). This difference is rather difficult to explain as all three studies contain a large sample of complex spike units (> 300). One of the reasons for this difference might be the different criteria for classifying a unit as place cell. In the paper by Scott et al. (2011) an age-independent, fixed arbitrary threshold for the coherence of the locational firing is used. Langston et al. (2010) and Wills et al. (2010) both use the 95^{th} percentile of an age-matched null-distribution for spatial information based on spike-shuffled data. The idea here is to dissociate the spike train from the animals' position in the recording environment and obtain an objective threshold for spatial information, above which it becomes very unlikely to find certain spatial information scores by chance. Only data exceeding this threshold is then considered for further analysis. A fixed and arbitrary cutoff value (as in Scott et al. (2011)) might bias the data towards selecting only the 'best' place cells in younger animals. A threshold derived from the actual data and depending on the age of the animals might be more feasible as a selection criterion during a time period when place cells undergo a functional maturation towards adult-like levels. Moreover, Scott et al. (2011) do not smooth the rate maps of recorded place cells which might account for the much lower inter-trial stability reported in this paper (ca. r=0.3 at P30) compared to the other two studies (ca. r=0.6 at P30). The reason for this rather protracted development of place cell firing described by Scott et al. (2011) remains unclear. One general caveat of Scott et al. (2011) is, that no recordings from adult animals were included, and thus it not possible to assess whether the

above mentioned methodological differences generally lead to different results even for adult data. Finally there is some preliminary evidence for phase precession in place cells recorded on a linear track as early as P17-18 (Langston et al., 2010; Wills et al., 2010).

As a summary it seems safe to conclude that neither the stability nor the quality of the spatial tuning of place cells in CA1 (but see Langston et al. (2010) for a different report on the latter measure) is preconfigured to adult levels by P16. The time line for the functional maturation of place cell properties approximating adult levels between the end of the second and fourth week described in the two of the studies (Langston et al., 2010; Wills et al., 2010) generally matches well with the behavioural development of young rats (see section I.4).

I.3.3.2 Head direction cells in development

The data on HD cells is the most consistent across all studies which include recordings of this cell type in young rats (Langston et al., 2010; Wills et al., 2010). Both report significant proportions of head direction cells with adult-like properties from the earliest time point of recordings (P15 in Langston et al. (2010), P14-16 in Wills et al. (2010)).

Neither the percentage of directionally modulated units nor the quality of the directional tuning and inter-trial stability of directional modulation of individual cells increases significantly with age. Furthermore Wills et al. (2010) report coherent rotations of head direction cell ensembles in two different environments as early as P16. It is noteworthy that head direction cells were recorded from different brain regions in the individual studies (PrS and PaS in Langston et al. (2010), MEC and PrS in Wills et al. (2010)) which implies that the neuronal network that encodes heading direction is more or less fully mature throughout the hippocampo-parahippocampal system by the end of the second week at P14.

I.3.3.3 Grid cells in development

Both studies that recorded grid cells from MEC between P16-28 or P16-34 respectively (Langston et al., 2010; Wills et al., 2010) obtained similar results, although the respective authors interpret the data in different ways. Wills et al. (2010) report the first occurrence of adult-like grid cells around P20-21, while Langston et al. (2010) report grid cell firing as early as P16. However, looking at the data of both papers it becomes clear that the respective results are actually quite similar. The firing of putative grid cells in Langston et al. (2010) before P21 mostly lacks the strict periodicity of adult grid cells and the authors acknowledge in their discussion that it is not until the end of the third week that this periodicity occurs. As far as the quantification of the quality of grid cell firing is concerned, both studies come to comparable timelines. Wills et al. (2010) report a rapid and rather abrupt increase in the percentage of grid cells from the whole cell population, as well as an increase in stability of grid cell firing across trials between P20-24, when adult levels are reached; see also Wills et al. (2012). In Langston et al. (2010) this development progresses until the end of the fourth/beginning of the fifth week and seems to be more gradual. It is noteworthy that in this study no increase in the proportions of grid cells is reported between P16-35, but a general significantly higher proportion for adult animals.

The delayed emergence of grid cells compared to place cells challenges the view of the importance of grid cell input for the spatial firing of place cells (Blair et al., 2008; Solstad et al., 2006). It rather suggests that other spatially modulated cell types in the hippocampo-parahippocampal network like e.g. border cells (Solstad et al., 2008) or boundary vector cells (Lever et al., 2009) might play a role in stabilising the location specific firing of place cells in early development.

I.3.4 Summary of spatially modulated cells

This section described in great detail the properties and determinants of place cell firing in the CA3 and CA1 area of the hippocampus of adult rats. Place cell firing correlates best with a certain location in physical space and is under the influence of external and internal sensory cues. Place cells seem to be largely configural, meaning that they only require parts of the original sensory input to re-activate a representation of an environment. Different environments are represented by the activity of different place cell ensembles. Furthermore the hippocampo-parahippocampal network also contains a head direction signal (HD cells in PoS and MEC) as well as a potential metric signal (grid cells in MEC, PrS and PaS).

All these cell types emerge at different time points during the postnatal development, and show a different time course of their functional maturation. HD cells are present first (P14-15) and seem to be adult-like from the time point of their emergence, while place cells emerge slightly later and show a gradual maturation between P16-35. Grid cells only emerge around P21 and show a very rapid and abrupt functional maturation until P24 (see Figure I-7 for a schematic overview of the emergence of the different cell types).

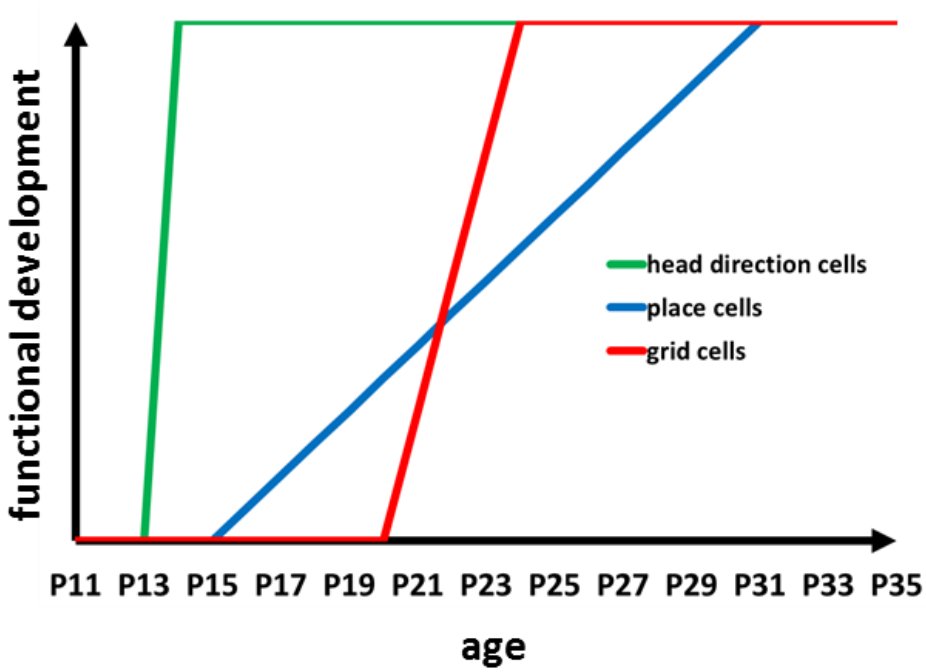


Figure I-7: Schematic overview of the timeline of the functional emergence of head direction cells (green), place cells (blue) and grid cells (red) between P11-35. Figure indicates period from emergence until when approximately adult-like levels are reached. Scheme is based on results from Wills and colleagues (Wills et al., 2012, 2010) and Langston et al. (2010).

I.4 Developmental milestones

The preceding chapters highlighted the postnatal functional and anatomical development of the hippocampal system in rats. As the experiments reported in this thesis are aimed at shedding light on the functional development of the neuronal systems which underlie spatial navigation and memory, it is also important to present a timeline of the behavioural development of young rats.

Rats are altricial animals, as they are born in a rather premature state and only develop the adult behavioural repertoire postnatally during a prolonged period spanning several weeks. A broad overview of the development of the motor and sensory systems will also be provided here as: i) spatial navigation requires active locomotion and, ii) place cell firing is sensitive to the presence of environmental sensory stimuli (see section I.3.1.6). A detailed description of the development of the neuronal systems underlying locomotion and sensory processing are beyond the scope of this thesis, which is why the emphasis will be on the behavioural aspects/expression of these systems.

The discussion will mainly focus on the development of hippocampal-dependent skills/behaviours with reference to the time points when adult-like performance is reached.

I.4.1 General overview of behavioural development

During the first 10 to 14 days of life, rat pups only rarely leave the nest for extended periods of time, and spend nearly all their time in the litter huddle (Bolles and Wood, 1964; Gerrish and

Alberts, 1996; Loewen et al., 2005). The main activities rat pups engage in during that time period are sleeping and nursing.

A variety of social behaviours like e.g. play-fighting and social grooming (i.e. grooming of littermates) start to emerge during the third week (Bolles and Wood, 1964; Thiels et al., 1990).

In terms of their consummatory behaviour rat pups first show an interest in solid food (i.e. typical laboratory rat chow) and water around P15-16, but they still get nursed regularly by their mothers until the age of weaning (Bolles and Wood, 1964; Thiels et al., 1990). Weaning is typically induced at P21 in laboratory rats (Bolles and Wood, 1964; Moye and Rudy, 1985; Schenk, 1985), which probably does not reflect the situation in wild rats as rat pups continue to suckle until P34 if they are kept with their mothers (Thiels et al., 1990).

I.4.2 Development of motor behaviour

On the day of birth the behavioural motor repertoire of rats is only poorly developed. Coordinated, quadrupedal locomotion is not yet present, but the animals are however capable of certain maternally directed orienting behaviours like turning upside down, vocalising or attaching to a teat, all of which tend to occur in a systematic sequence (Polan and Hofer, 1999; Polan et al., 2002).

In terms of the development of locomotion two articles offer a particularly detailed qualitative description of when a specific set of motor skills develop, both under standardised laboratory testing conditions (Altman and Sudarshan, 1975) and under more 'natural' conditions in the home cage (Bolles and Wood, 1964). Both studies describe the gradual emergence of adult-like locomotion during the first three weeks of a rat's life. During the first week relatively simple

motor behaviours prevail. Rat pups show a righting reflex (i.e. turning around on their feet when placed on their back) already at P0, although the speed and level of coordinated movements increases with age until P10-15 when adult speed of righting (< 1 s) is reached. Together with the righting reflex, pivoting movements develop. These are circular body movements due to activity of the front limbs and parallel inactivity of the hind limbs. The first pattern of true locomotion that emerges is crawling, and this becomes apparent during the middle to end of the first week. Full, species-specific, quadrupedal walking can first be observed around P10, although it is often sluggish and rather a mix of walking and crawling at that age. Around P14 quadrupedal walking occurs frequently and by P20-21 adult patterns of walking are reached. Quadrupedal walking develops with a front-to-back limb pattern, meaning that the former develops earlier, resulting in an initial paddling-like movement pattern (Westerga and Gramsbergen, 1990). From my own personal observations, the locomotion of rat pups looks indistinguishable from that of adult rats around P19-21.

One important point to note is that behaviours like e.g. adult-like walking can actually be expressed much earlier by rat pups in certain aversive conditions (like walking on a refrigerated surface by P4) than they occur spontaneously (Altman and Sudarshan, 1975).

I.4.3 Development of sensory processing

Just as the motor system, the rat's sensory systems are very rudimentary at birth. For instance, rats are deaf, their eyelids are sealed and their whiskers are immobile when they are born.

I.4.3.1 Olfaction

The olfactory system is the first sensory system to emerge. Already shortly after birth (P3-5) rat pups can discriminate between different odours and show a preference for nest shavings over e.g. lemon scent (Cornwell-Jones and Sobrian, 1977). Moreover if rat pups (at P2) are exposed to an odour and nausea is induced by Lithium-chloride injections soon after, they show a clear aversion to this odour at P8. This indicates that not only can these animals detect the odour at P2, but they also possess the ability for associative olfactory learning very early in development (Rudy and Cheatle, 1977).

I.4.3.2 Touch

Whisking behaviour (active vibrissae movements) in rodents plays an important part in tactile perception (Zucker and Welker, 1969) and is involved in a variety of behaviours such as surface or object detection as well as social behaviours (for an extensive review see Ahl (1986)).

Whisking in rat pups emerges in parallel with sniffing between P11-13 and its frequency and amplitude increases until around P28 when adult levels are reached (Landers and Zeigler, 2006; Welker, 1964). In a recent study it was shown that contact-dependent modulation of

whisking shows a gradual emergence and maturation between P11 and P17, and furthermore that whisking behaviour develops in parallel with the motor systems (on behavioural level) (Grant et al., 2012).

I.4.3.3 Hearing

The auditory system of infant rats is not functional until at least P8-9, as this is the time point when cochlear microphonic potentials can be observed for the first time from the round window of the inner ear in response to sound stimulation (Crowley and Hepp-Raymond, 1966; Uziel et al., 1981). By P11-12 the first action potentials can be recorded from the vestibulocochlear nerve (Uziel et al., 1981). By P14 rat pups can already discriminate between sounds differing by only 200 Hz (Rudy and Hyson, 1984) and also their ability to learn the association between a sound and a sucrose reward emerges around this time point (Hyson and Rudy, 1984). By P17-18 young rats can differentiate between two sounds of different frequencies in a differential appetitive classical conditioning paradigm (Rudy and Hyson, 1984).

I.4.3.4 Vision

Vision is the last sensory system to emerge with the opening of the eyes usually occurring between P14-17 (Altman and Sudarshan, 1975; Bolles and Wood, 1964; Fagiolini et al., 1994; Foreman and Altaha, 1991; Moye and Rudy, 1985; Prévost et al., 2010; Routtenberg et al., 1978). Electrophysiological recordings from primary visual cortex (V1) reveal that neurons already show adult-like spatiotemporal tuning functions to sinusoidal gratings approximately 48 hours after eyelid opening (Prévost et al., 2010). However, in a more detailed study

reporting extracellular recordings from the binocular portion of V1 (OC1B), Fagiolini et al. (1994) showed that the visual system undergoes protracted maturational changes, which continue until P45. In their article, the authors report that the animals opened their eyes at P14-15 and it was not until P19 that the eye optics became totally clear. Adult-like responsiveness of cells in OC1B to moving and flashing visual stimuli was reached by P23, while by P30 adult proportions of orientation selectivity (i.e. cells that preferentially respond to a visual stimulus at specific rotational angles and directions of a moving stimulus) are reached. Particularly relevant to the results reported in this thesis are findings related to the development of visual acuity. Visual acuity in rat pups increases quickly between P19 and P30 and adult levels are reached around P40-45 (Fagiolini et al., 1994).

Moye and Rudy (1985) showed, through behavioural testing, that while P15 pups are able to detect a visual stimulus (flashing light) it is only at around P17-18 that they are able to learn an association of the stimulus with an electric shock in a classical aversive conditioning paradigm.

I.4.4 Development of spatial navigation and hippocampal-dependent behaviours

I.4.4.1 Development of exploratory behaviour

Rat pups typically start leaving their nest around P14-16 (Altman and Sudarshan, 1975; Bolles and Wood, 1964; Gerrish and Alberts, 1996; Loewen et al., 2005) and the duration of these excursions increases steadily with age until the end of the third week when huddles are not formed anymore (Loewen et al., 2005). The emergence of an exploratory motive in rat pups

around the mid to end of the third week is further supported by the fact that young rats start to show a preference for a novel side of an environment at P19 (Goodwin and Yacko, 2004). At P24 rat pups show a preference for a novel object in an object recognition test and by P30 young rats have a memory of a location where they had previously encountered an object (Ainge and Langston, 2012). Both of these behaviours are present in adult rats (Dix and Aggleton, 1999; Ennaceur and Delacour, 1988) and the latter is impaired by cytotoxic hippocampal lesions (Mumby et al., 2002).

I.4.4.2 Development of path integration

Path integration or dead reckoning describes the ability of an animal to home back in a straight trajectory to a starting position (e.g. nest) after an excursion, taking into account only the distance and directions traversed during the outbound journey (Etienne and Jeffery, 2004). For rodents it has been conclusively shown that these animals possess this ability (Maaswinkel et al., 1999; Mittelstaedt and Mittelstaedt, 1982; Whishaw and Maaswinkel, 1998; Whishaw and Tomie, 1997) and that this ability depends to some extent on a functioning hippocampus (Maaswinkel et al., 1999; Whishaw and Maaswinkel, 1998).

Rat pups, when placed on a circular platform in between their home cage and an empty cage, already show a pivoting orienting response towards the home cage as early as P3 and fully and reliably orient towards the home cage at P8 (Altman and Sudarshan, 1975). At this age they also can home physically to the home cage from an adjacent empty cage (Altman and Sudarshan, 1975).

In terms of path integration there is good evidence that rat pups have such abilities from the start of nest egression, and at least over the environment used in the study (Ø 1.5 m circular

platform) this seems to be fully functional from P16 (due to the increasing length of the outward journeys it is difficult to directly compare path integration of pups at different ages) (Loewen et al., 2005).

I.4.4.3 T-maze in development

Although very simple in design the T-maze offers fairly easy assessment of hippocampal function, using a variety of different tasks, measuring spontaneous alternation as well alternation in a delayed forced choice design (Deacon and Rawlins, 2006). As the name implies, it consists of a T-shaped narrow arm maze with the starting point located in the stem of the 'T' and two goal arms which extend from the T-junction. In a typical experiment, the animal is released from a start box at the bottom of the start arm and, depending on the task, one or both goal arms are open. By offering the animal access to both goal arms, rates of spontaneous alternation (unrewarded) between trials can be assessed as well as alternation (rewarded) in a delayed forced choice design. Here a trial consists of two runs: in the first run only one goal arm is open while in the second both are opened, but only entry to the previously unvisited arm is rewarded. By varying the delay between these two runs this allows an assessment of working memory function. Finally, reference memory can be tested in a T-maze as well, by only rewarding entrance to one particular goal arm throughout the behavioural training. For adult rats it is well established that hippocampal lesions impair spontaneous alternation (Johnson et al., 1977) as well as performance on the forced choice version (Dudchenko et al., 2000).

Kirkby (1967) reported a gradual increase in the average spontaneous alternation rates in a T-maze between P20 and P80, with P20 animals performing at chance levels. Douglas et al. (1973) report that, on average, rat pups reach a criterion (75% alternation across 20

consecutive trials) between P23-33 (‘normal developers’), but a small fraction of animals only reaches this criterion between P61-65 (‘late developers’).

When rat pups are rewarded for alternating in a free choice as well as in a forced choice version of the T-maze, reliable high rates of alternation (70%) can be observed after 20-30 trials in P21 animals (Green and Stanton, 1989). At P15, animals usually show long chains of persevering on one side instead of alternating even when rewarded, and animals at this age furthermore do not learn the forced choice version of the task (Green and Stanton, 1989). In contrast to this, animals at P15 can already solve a reference memory version of the T-maze task (Green and Stanton, 1989). It is however noteworthy that even at P33 rats are still more likely to make errors and less likely to produce long chains of errorless trials in the reference memory T-maze task compared to adult animals (Bronstein and Spear, 1972).

I.4.4.4 Water maze in development

The Morris water maze (Morris, 1981) is one of the most widely used behavioural paradigms to assess spatial learning and normal hippocampal function. In this task, rats need to learn the fixed location of a submerged platform in a pool of water, with respect to the room cues (‘hidden platform’). This task is known to depend on hippocampal function in adult rats, as lesions (Morris et al., 1982) as well as pharmacological inactivation (Riedel et al., 1999) of the hippocampus abolish the ability to solve this task. Furthermore, if a salient cue is placed near the platform (‘cued version’) the ability to find the platform is independent of normal hippocampal function (Morris et al., 1982).

In the developmental literature, the two major points of debate regarding the emergence of navigational skills as assessed by water maze tasks are: i) the exact time point, during

development, when place navigation emerges in young rats; and ii) the question of whether cued navigation emerges in parallel or earlier than place navigation. The exact time point when young rats are able to solve the hidden platform version of the water maze varies between P19-21 (Akers and Hamilton, 2007; Brown and Kraemer, 1997; Brown and Whishaw, 2000; Rudy et al., 1987) and P28 (Schenk, 1985). This disparity in results is most likely due to methodological differences between studies, regarding e.g. training regime (i.e. one day vs. several days and massed vs. spaced training design) or active body temperature control by the experimenters (i.e. absent or present).

The exact time point when rat pups are able to locate the platform when a proximal cue signals its location also differs slightly across studies, with different studies reporting P16-17 (Akers et al., 2011), P18 (Rudy et al., 1987) or P19-20 (Brown and Whishaw, 2000). Furthermore, the latter study (Brown and Whishaw, 2000) reports a parallel emergence of the ability to solve the cued and the hidden platform version of the water maze task, while the other two papers show a sequential emergence with cued navigation, preceding the ability to solve the hidden platform variant.

Systematic one day testing of rat pups between P16 and P24 on the cued version demonstrates that already at P16-17 there is a reduction in escape latencies across training trials (Akers et al., 2011). Interestingly, the analysis of the probe trials after this cued training shows that at least at P16 no spatially biased search pattern is apparent in rat pups, while at P17 the first signs of a directional bias emerge (Akers et al., 2011). This points out to a sequential development of the ability to solve both the cued and the place version of the water maze task. Although generally believed to be independent from hippocampal function, the exact time point when the ability to locate a cued platform first emerges is of some significance for the work presented in this thesis. This allows making some assumptions regarding the visual acuity of developing rats, in that it shows when visual acuity is sufficient

for the animal not only to perceive a proximal visual cue, but also to use this information to guide their behaviour.

I.4.5 Summary of developmental milestones

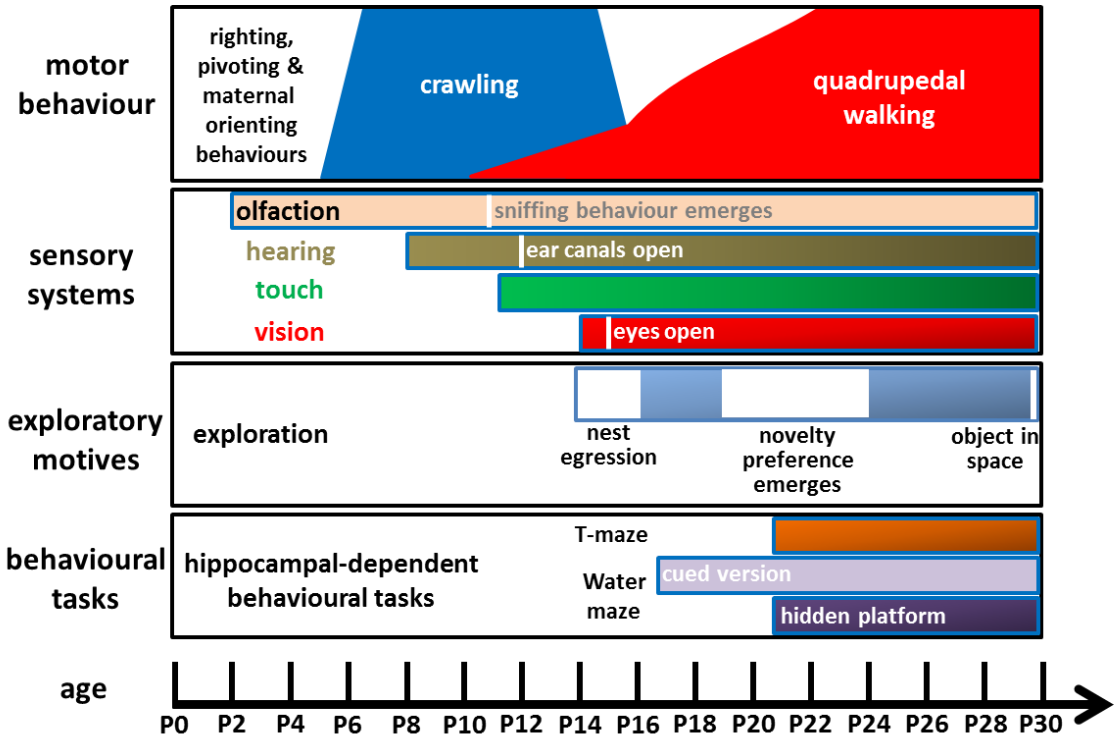


Figure I-8: Overview of the emergence of motor behaviours (top box), sensory systems (2nd from top), exploratory motives (2nd from bottom) and the ability to solve hippocampal-dependent tasks (bottom). The age of the animal is indicated at axis at the bottom.

This section described the emergence of the behavioural repertoire of adult rats in developing rat pups during the first four to five weeks of postnatal life. At birth, both the motor and sensory systems of rat pups are poorly developed. In terms of motor behaviour, rat pups show a rapid maturation during the third week and by the end of the fourth week their general motor abilities are more or less adult-like. The different sensory systems emerge in the order of olfaction (first week), hearing (end of second week), touch (end of second week), and vision (beginning of third week). All modalities show a functional maturation and become adult-like at different time points between ca. P21-45.

Rat pups first leave their nest around P15-16 and subsequently show the emergence of an exploratory motive, typical of adult animals. During the fourth week rat pups develop the ability to solve different behavioural tests, which are known to depend on the integrity of the hippocampus in adult rats (e.g. T-maze and water maze).

This behavioural development seems to match well with the functional development of spatially modulated cells in the hippocampo-parahippocampal network, which are thought to provide the brain systems underlying spatial navigation.

Figure I-8 gives a schematic overview of the emergence of all the above mentioned behaviours and systems.

I.5 Rationale for experiments in this thesis

Previous work has described the functional development of place cell firing in the hippocampus of rat pups in a familiar environment (Langston et al., 2010; Martin and Berthoz, 2002; Scott et al., 2011; Wills et al., 2010). As described in great detail in the introduction of this thesis, place cells in adult rats are under some control of sensory stimuli. Since the sensory systems of rat pups emerge postnatally at different time points and show marked differences in the time course of their functional maturation, it is so far unclear whether sensory integration in place cells might also function differently during this time period.

That is why the experiments of this thesis are aimed at shedding light on the responses of place cells, when certain sensory cues in a familiar environment are manipulated. These will be local olfactory/tactile cues on the walls and floor of the environment, as well as the presence/absence of visual information. The initial working hypothesis for this thesis was, that early in development place cells might not be configural and much more bound to individual local olfactory/tactile cues, since olfaction is the first sensory modality to emerge and mature. Instead, for vision it was hypothesised, that an influence of this modality might not be present initially and only develops later in development in parallel with the maturation of this sensory system.

II Methods

II.1 Animal housing and husbandry

II.1.1 Housing

All animals involved in breeding (studs, dams and litters) were of the Lister-hooded strain and kept on a 12:12 hour light:dark schedule with lights off at 12:30 pm. Studs were always single-housed while females were group-housed (2-4 animals) until the first breeding, after which they were also single-housed. Litters remained with their respective mothers until the age of weaning (P21). All animals had *ad libitum* access to food and water. All experiments were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986.

II.1.2 Adult controls

Adult control animals were male Lister-hooded rats aged between 3-6 months at the time of surgery. These animals were group housed (2-4 animals) until the day of surgery, after which they were single-housed. They had *ad libitum* access to water and food until 1 week after surgery, after which they were maintained on 90% of their free-feeding weight. A total of 10 control animals were used in this study, weighing between 300-400 g at the day of surgery. All experiments were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986.

II.1.3 Breeding

A designated dam was put into the cage of a designated stud for 7 days. Thereafter the dam was put into a 'breeding cage' with plenty of bedding (tissue paper, Tork®advance) where she remained until the pups were weaned. This cage was cleaned (i.e. replacing saw dust and bedding) once a week until the animal looked heavily pregnant. Pregnant dams were checked daily for a litter around 5-6 pm and if one was present this day was deemed the day of birth (P0). Between P3-5 litters were culled to a standard size of 8 male animals (if possible). If litter size was less than 8 animals all animals were kept and if less than 8 males were present some female animals were kept as well to make up for a total of 8 animals. Litters remained with their mothers until weaning which happened typically at P21. However, if rat pups were implanted with recording electrodes at P20-21 weaning was usually postponed until P22-23. After a litter was delivered the home cage was first cleaned (i.e. replacing saw dust and bedding) at P10, and then once a week from there on.

In this study a total of 7 studs and 15 dams were used for breeding. This yielded a total of 16 litters of which 1 contained less than 8 animals (this litter contained 7 animals). The remaining litter sizes varied between 9-18 animals (overall mean: 13±4 animals). Two litters consisting of 8 animals were bought directly from a company and no information on original litter size was available. The age range of studs was 5-11 months and that of dams 4-10 months. An individual dam could have a total of 3 litters with a period of at least 4 weeks between weaning of the last litter and the following pairing with the designated stud. In general dams had to weigh at least ca. 200 g when used for a breeding for the first time.

A total of 27 rat pups (from 16 litters in total) across an age range of P12-22 and weighing between 24-55 g at the day of surgery were implanted with a microdrive. This is the number of

animals where at least one place cell was recorded at some point in an experiment (3 animals were typically implanted per litter).

II.2 Surgery

II.2.1 Rat pups

Surgical procedures for adult controls were similar to Cacucci et al. (2004) and those for pups similar to Wills et al. (2010).

Rat pups were given a subcutaneous injection of buprenorphine (Alstoe Animal Health; York, UK) at a concentration of 0.15 µg/g body weight 30-50 min prior to surgery. During this time they were returned to their home cage. Animals were then anesthetised using 3% isoflurane (Abbott; Maidenhead, UK) and O_2 (3 L/min). During surgery the concentration of isoflurane was gradually lowered to 0.75-1% (typically reached half way through surgery). Animals were mounted on a stereotaxic frame (custom made model) and chronically implanted with microdrives loaded with 4 tetrodes (HM-L coated 90%-platinum-10%-iridium 17 µm diameter wire; California Fine Wire; Grover Beach, CA, USA) aimed for the left hippocampus.

Tetrode tips were platinum plated in 1:9 0.5%-gelatine:Kohlrausch solution until the impedance on every channel was brought down to 100-200 kΩ (Merrill and Ainsworth, 1972). Plating was usually done the day before surgery, but never more than 3 days prior to implantation of the microdrive.

A central incision was made in the skin of the head to expose the skull, such that bregma and lambda were identifiable and sufficient space for the insertion of holding screws and microdrive was available. In total 7 jeweler's screws (length: 0.0625 inches; Small Parts; Logansport, USA) were spread across the exposed skull (2 in frontal plate, 2 in parietal plate opposite to microdrive, 2 in occipital plate and 1 on parietal plate posterior to microdrive) to

secure the microdrive. After tetrodes were lowered into the brain, holding screws, the exposed skull and the 'feet' of the microdrive were covered with dental acrylic (Simplex rapid; Associated Dental Products Ltd.; Swindon, UK), which held the drive in place and closed the wound on the animal's head.

The following coordinates were used for pup surgeries:

- In a subset of animals (n=5) the electrodes were aimed at anterior levels of the hippocampus (CA1/CA3) using the following coordinates: 1.6 - 2.0 mm AP (anterior-posterior), 1.2 - 1.5 mm ML (medial-lateral), 1.5 mm DV (dorsal-ventral).
- For the remaining animals (n=22) the following coordinates aimed at more posterior levels of CA1 were used: 3.0 – 3.3 mm AP, 1.9 - 2.0 mm ML, 1.5 - 1.7 mm DV.

The different AP and ML coordinates are due to differences in the length of the bregma-lambda axis. In general a length of 7.4 mm was used as standard size and coordinates were updated accordingly.

To minimise heat loss during surgery a water-powered heat pad (UNO; Zevenaar, Netherlands) at a temperature of 38°C was used, onto which the animals rested during surgery. Additionally, surgeries were conducted as fast as possible and never exceeded 2.3 hours of anesthesia.

After surgery was finished, animals were put next to a heat pad (Snugglesafe®; http://www.zooplus.co.uk) inside a recovery cage, and once they recovered from anesthesia they were returned to their home cage. To avoid the mother 'attacking' the microdrive or not accepting the pup, a 'mock microdrive' (containing the plug for the headstage, blue tak® (Bostik, Stafford, UK), dental acrylic and a metal cannula) was put inside the home cage one day before surgery.

II.2.2 Adult controls

There were some minor differences for the surgical procedures on adult controls. For analgesia animals received a subcutaneous injection of caprofen (Pfizer; Sandwich, UK) at a concentration of 5 µg/g body weight 5-10 min before surgery. They also received a subcutaneous injection of enrofloxacin (Bayer; Newbury, UK) at a concentration 25 µg/g body weight 5-10 min before surgery. For post-operative analgesia they received 1 cube (ca. 10 ml) meloxicam jelly per day which corresponds to a dose of 1 µg/g body weight of meloxicam (Boehringer Ingelheim; Ingelheim, Germany) for three days after surgery. The coordinates of all adult implants were, as measured from bregma: 4.0 mm AP, 2.5 mm ML and 1.5 mm DV. All implants were aimed at the left hippocampus, apart from 2 animals which received double implants in the left and right hippocampus, using the same coordinates for both hemispheres.

II.3 Data collection

The day after surgery screening for hippocampal complex spike cells began for implanted rat pups. Tetrodes were lowered ventrally in 62.5 or 125 µm increments (microdrives in adult animals could be lowered in 25 µm increments) until physiological indicators typical of CA1 were present. These were ripple oscillations (100-200 Hz with duration of 20-30 ms) and/or the presence of complex spikes. To avoid instability of the recording due to tetrode movements at least ca. 1 hour had to elapse between the last movement of the tetrodes and the start of an experiment.

Trial data was recorded using an Axona dacqUSB data acquisition system (Axona Ltd.; St. Albans, UK). For single unit recordings, signals were amplified (10-20K) and band-pass filtered (360 Hz - 7 kHz) and whenever a signal exceeded a set certain tetrode specific threshold (typically about 65-75% of the maximum signal amplitude on a given channel/tetrode) it was recorded. Each tetrode channel was monitored at 50 kHz and individual action potentials/signals were stored as 50 points across a 200 µs pre- and 800 µs post-threshold time period. Whenever a signal exceeded the threshold on any tetrode channel the signal was recorded on all tetrode channels. Units recorded at different days or after the tetrodes were lowered were always treated as different cells.

The hippocampal EEG was recorded by band-pass filtering the signal between 0.34-125 Hz and a 50 Hz notch filter at a sample rate of 250 Hz. EEG signals were typically amplified 8-15K. Usually the EEG from at least 2 different tetrodes was recorded in parallel.

The position and head orientation data was recorded via a camera positioned above the middle of the environment using two different size LEDs attached to the head of the animal at

a fixed distance and a fixed orientation relative to the animal's head. This allowed tracking of position as well as heading direction. Positions were sampled continuously at 50 Hz.

The data collection did not differ between rat pups and adult rats except that adult rats were given a seven day recovery period after surgery before screening started (see Figure II-2 for a schematic overview of recording setup).

II.4 Behavioural paradigm

The experiments in this thesis consisted of exposing animals (rat pups and adults) to a variety of different open field enclosures, differing in their sensory properties from a familiar environment that served as a baseline condition. The sensory properties of the familiar environment were kept as constant as possible and the probe trial environments differed more or less strongly with regards to these properties.

II.4.1 Apparatus

II.4.1.1 Holding platform

During screening for place cells and in between recording trials the animals rested on a holding platform (dimensions: 35x35x30 cm) with a heat pad (Snugglesafe®; www.zooplus.co.uk) that was constantly monitored and replaced with a new one once it felt cold. For very young pups (< P18) the duration of screening sessions was kept as short as possible (ca. 20-30 min).

II.4.1.2 Familiar environment

The familiar environment consisted of a grey wooden box with dimensions 61.5x61.5x51 cm. The floor consisted of a sheet of transparent Perspex® (Amari Plastics; Weybridge, UK) on a black wooden table top. Surrounding the box were several custom made distal cues (posters with random high contrast pattern) attached to the room walls. No polarising cues were

attached to the box walls inside the environment. For a given litter the floor in the familiar environment was never wiped/cleaned in between trials. Only urine puddles and fecal pellets were removed from the environment. Once testing was finished for a given litter the floor of the familiar environment was thoroughly cleaned with soapy water.

II.4.1.3 Probe trials

'New walls'

The 'new walls' environment consisted of the familiar environment except that the walls were replaced for a visually identical replica. This was done to control for olfactory/tactile cues on the environment walls. These walls were different from the ones used for the 'new floor & new walls' environment.

'New floor'

The 'new floor' environment consisted of the familiar environment except that the floor was replaced for a visually identical replica. This was done to control for olfactory/tactile cues on the environment floor. The 'new floor' was always cleaned with soapy water in between animals and recording trials.

‘New floor & new walls’

The ‘new floor & new walls’ environment consisted of the familiar environment, except that the walls and floor were both replaced for visual identical replicas. This was done to control for olfactory cues on both the environment walls and floor. The floor was the same one as in the ‘new floor’ environment and always cleaned with soapy water in between animals and trials. The walls were a different replica as the ones used for the ‘new walls’ environment.

‘Familiar dark’

This refers to recordings inside the familiar environment in absolute darkness to test for the effect of removal of visual input on spatial representations.

‘Novel environments’

The novel environment was located in a different position in the experimental room and surrounded by black curtains so the animal could not see the rest of the room when inside. Two sets of novel environments were used.

The ‘novel light’ environment consisted of a square shaped ‘morph box’ similar to the one used in Wills et al. (2005) with dimensions 61x61x48.5 cm and a plastic floor (different to the material from the familiar environment). The ‘morph box’ was made out of 32 pieces of cable trunking and the inside was covered with brown packing tape. One white A0 card was positioned outside the environment (north wall) serving as the only polarising visual cue.

The ‘novel dark’ environment consisted of the walls from the ‘new floor & new walls’ environment, but with a large piece of cardboard attached to the whole area of the north wall. The floor was a piece of black painted wood. For a subset of animals the ‘morph box’ was used as the ‘novel dark’ environment. These animals were not recorded in ‘novel light’ conditions. Although some of the animals had experienced the walls before, the combination with the card board and the different completely novel floor made this environment very dissimilar to the familiar context.

Both novel environment floors were cleaned with soapy water in between animals and recording trials.

II.4.2 Dark recordings

For recordings in the dark the familiar environment (‘familiar dark’) or one of the novel environments (‘novel dark’) was used and the recording room was made pitch black by switching off all the lights. The lights were always switched off at least 30 s prior to the start of a recording trial, while the animal was still on the holding platform and always only switched back on again after the animal was returned to the holding platform after the trial had finished. The experimenter wore night vision goggles (model: Yukon NVMT 1 2x24; Laser King YukonScopes; Fremont, Ohio; USA) during recording trials in the dark. Near the recording environment the infrared illuminator of the goggles was always switched off.

II.4.3 Adult rats

Control recordings from adult rats were conducted in a different experimental room, but using the same types of environments in terms of dimensions and material for the familiar, the 'new walls', the 'new floor', the 'new floor & new walls' and the 'novel light' environment. The 'novel dark' environment consisted of a black wooden box with a rough texture (dimensions: 61x61x60 cm). For adult controls the position of the novel environment was in a different location inside the same room as the familiar environment, but was not surrounded by black curtains.

To assess the relationship between average place cell stability and field-to-wall distance in a 'large' environment for the adult control group the following environment was used. The dimensions of the box were 122x122x51 cm. It had wooden walls (black) and a floor made out of a thick plastic sheet. It was located in the same room as the familiar environment. This experiment served as an additional control to understand whether the absence of a correlation between the two measures (place field stability and field-to-wall distance) in adult animals is potentially due to the larger body size-to-environment ratio.

II.5 Recording procedure

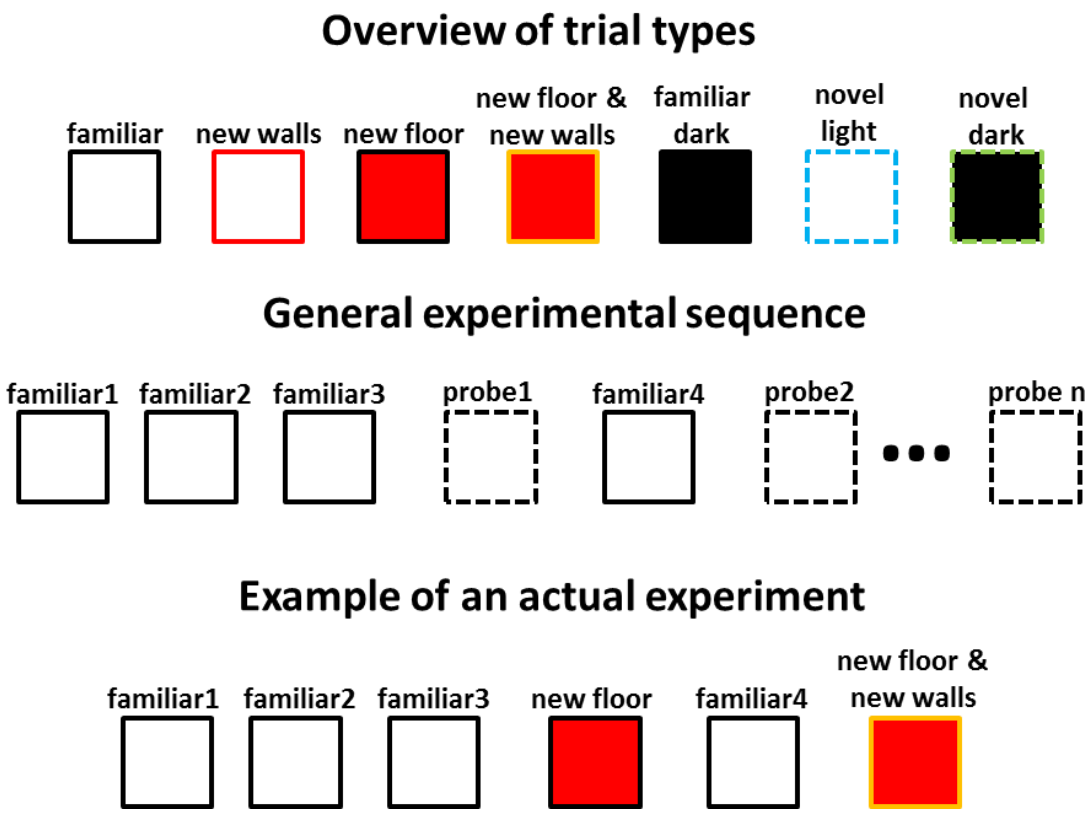


Figure II-1: Schematic overview of experimental design. Top row shows different trial types (this scheme will be used throughout this thesis). Middle row shows general experimental sequence and bottom row gives an actual example of a typical experimental trial sequence.

II.5.1 Rat pups

Irrespective of whether place cells were present or not each animal received at least 2 recording trials per day inside the familiar environment, starting one day after surgery for rat pups. Once place cells were present a series of probes was started (see Figure II-1). Two to three trials in the familiar environment were run, followed by a sensory manipulation trial (any

of the probe environments), another ‘familiar’ and a final probe trial (different probe to first probe trial). This sequence of 5-6 trials was usually used in very young animals (> P19-20) to minimise the time the animals were separated from their litter. In older animals occasionally another ‘familiar’ and probe trial was run. In some cases the series was terminated after the first probe trial or only ‘familiar’ trials were run. It is noteworthy that the sequence of probe trials was not random. Usually the amount of change to the familiar environment was subsequently increased in a series of probes (i.e. e.g. probe1=’new floor’ and probe2=’new floor & new walls’ or probe1=‘familiar dark’ and probe2=’novel dark’).

A recording trial in any environment lasted 10-15 min depending on running speed and general coverage of the environment by the animal. Within one experimental series trial duration was always kept constant. Inter-trial interval was always 10 min. During the trials the experimenter moved constantly around the environment and occasionally put his hand inside to encourage exploration. Once rat pups were habituated to soy-based formula milk (conc.: ca. 0.16 g/ml; SMA; Maidenhead, UK) this was used as bait to encourage exploration. This typically was not possible until P19-20. To habituate pups to formula milk, a sample was placed in the animals’ home cage at P17-18 and additionally the mother was manually fed ca. 1 ml of soy milk per day. Implanted animals were also manually fed some milk in the experimental room.

II.5.2 Adult rats

The recording procedure for adult rats was generally similar to that for rat pups except for a few changes. Usually, the whole sequence of all probes was run in one single recording session interspersed with trials in the familiar environment. However, in some experiments only a subset of all probes was tested. Trial and inter-trial duration was always 10 min and to

encourage exploration bits of rice grains were randomly thrown into the environment by the experimenter during the recording trials.

Recording trials in the 'large box' environment were always run within a separate experimental series with the sequence familiar-familiar-large box-large box or familiar-large box-familiar-large box. Trials inside the large box lasted 30 min.

II.6 Data analysis

All data was analysed using Matlab R2011b (MathWorks®; Natick, USA) and Microsoft Excel®. Statistical analyses were performed using Matlab R2011b and SPSS v.20 (IBM corp. ®).

II.6.1 Cluster cutting

Cluster cutting of spikes was performed manually using the custom written analysis program Tint (Axona Ltd., St. Albans, UK). Spike clusters were separated on the basis of their different amplitudes on individual tetrode channels. Spike sorting quality was assessed by calculating L-ratio and isolation distance according to Schmitzer-Torbert et al. (2005). The multidimensional feature space for a given cluster consisted of the peak-to-trough amplitudes of the cluster spikes on all tetrode channels. There usually were 4 channels per tetrode, but in case of dead channels or in case one channel had to be sacrificed for the EEG recording, there were only 2-3. Both measures are derived from the squared Mahalanobis distance (D^2) in a feature space of the spikes on a given tetrode from the centre of a given spike cluster of that tetrode. Isolation distance of a spike cluster with *i* spikes is D^2 of the *i*th closest spike not belonging to that cluster. This D^2 corresponds to the radius of an ellipsoid around the cluster centre containing an equal amount of spikes belonging to the cluster and 'noise' spikes. Large values for the isolation distance of a spike cluster indicate a good separation of that cluster from other clusters/noise. L-ratio of a spike cluster is the sum of the probabilities for all spikes not belonging to that cluster that they actually should be part of it (probability of a spike belonging to a cluster is the inverse of the cumulative distribution function for a χ^2-distribution with as many degrees of freedom as dimensions in the feature space). The resulting value is then

normalised by the number of spikes in a given cluster to account for differences in cluster size. Thus, a low value for an L-ratio of a spike cluster indicates little contamination of the cluster with other spikes/noise.

For each cell a mean for L-ratio and isolation distance was obtained by averaging the respective values across the trials inside the familiar environment before any probe trial was conducted.

II.6.2 Rate map construction

Rate maps were constructed by binning the environment in 2.5x2.5 cm bins and firing rate in each bin was obtained by dividing the number of action potentials in that bin by the dwell time. Rate maps were smoothed using an adaptive smoothing algorithm (Skaggs and McNaughton, 1998; Skaggs et al., 1996). The width of the smoothing radius r centered on each bin was calculated using the following formula:

$$r \geq \frac{\alpha}{n\sqrt{s}}$$

where α=200, n represents the dwell time (in s) and s the number of spikes inside the radius. Once the smoothing radius was established for a given bin firing rate for that bin was set equal to n/s. This approach ensured that larger smoothing windows are applied to under-sampled parts of an environment, while smaller ones are applied to well-sampled parts. In general the smoothing radius with this method tends to approach the size of a standard 5x5 bin Gaussian boxcar filter for well-sampled data (personal observation). Positional maps were adaptively smoothed using the same set of smoothing windows as the corresponding rate maps.

Rate maps are presented as false colour coded auto-scaled maps as percentage of peak firing rate in 10%-increments, with progressively warmer colours representing higher firing rates (colour range is from dark blue (0-10% of peak firing rate) to red (90-100% of peak firing rate)). White bins represent unvisited positions inside the environment.

All subsequent data analyses were based on adaptively smoothed rate maps unless otherwise stated (see Figure II-2 for a schematic overview of rate map construction).

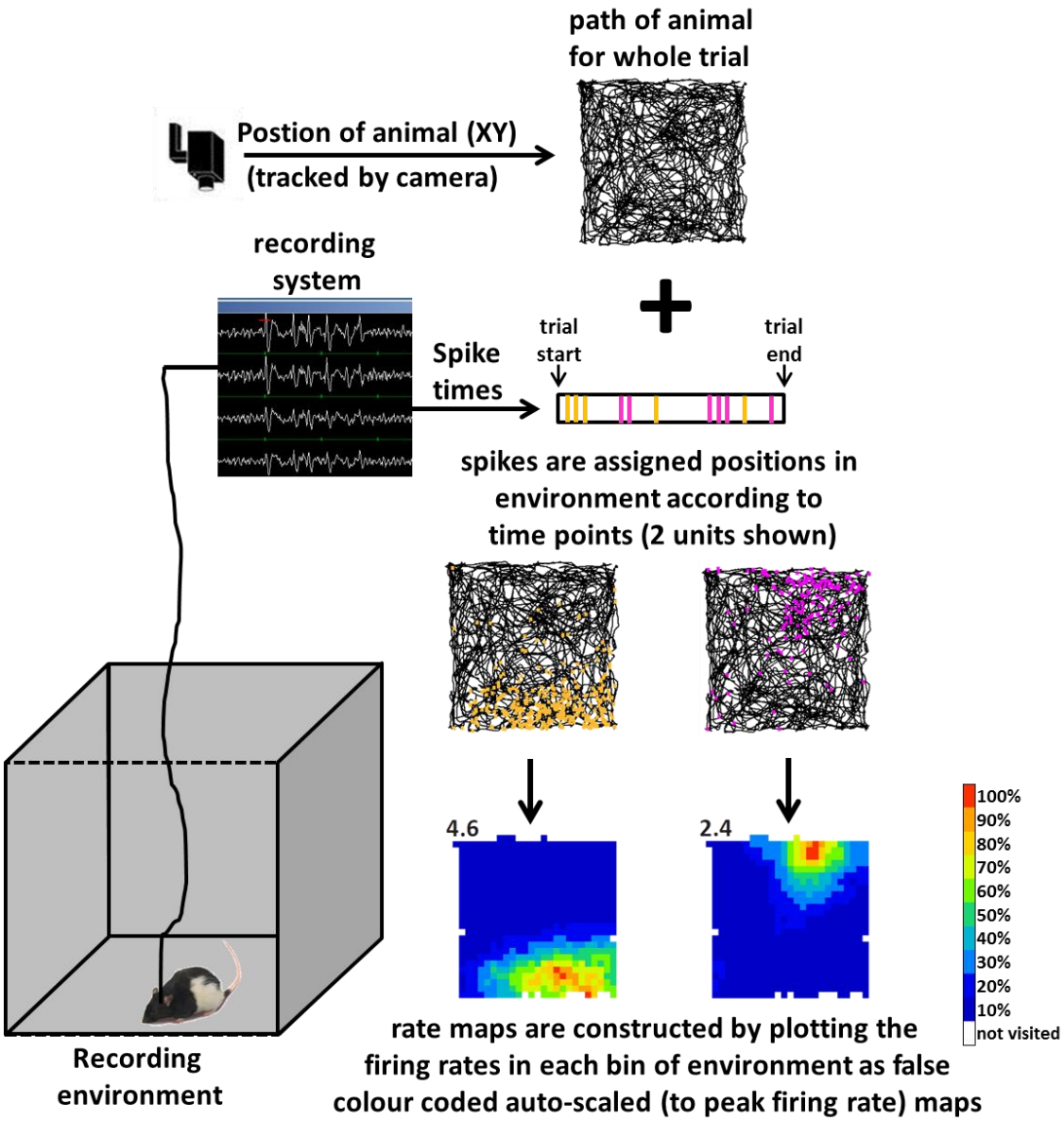


Figure II-2: Overview of recording setup and rate map construction. Depicted is the data acquisition procedure where an animal is exploring an environment (grey box) while the activity of neurons is recorded (recording system) and its position is tracked in parallel (camera). For rate map construction spikes are assigned positions on path and rate maps are obtained by plotting firing rates in each bin as false colour coded auto-scaled maps (see colour bar). Firing rate is scaled according to peak firing rate (peak firing rate (Hz) is indicated at top left corner of rate maps).

II.6.3 Single unit parameters (spatial tuning)

II.6.3.1 Firing rates

Mean firing rate was obtained by dividing the total number of spikes of a unit for a given trial by trial duration. Peak firing rate was the highest firing rate in any bin of a recording trial. Peak rate was obtained after adaptively smoothing the rate map.

II.6.3.2 Spatial information

Spatial information (Skaggs et al., 1993) is a measure to which extent the locational firing of a given unit can be used to predict the animal's position. It is measured in bits/spike and calculated using the following formula:

$$I(R|X) \approx \sum_{n} p(\overrightarrow{x_n})\, f(\overrightarrow{x_n}) log_2 \left(\frac{f(\overrightarrow{x_n})}{F} \right)$$

I(R|X) denotes the mutual information between firing rate R and position X, $p(x_n)$ is the probability for the animal being at location x_n, $f(x_n)$ is the firing rate at x_n and F is the mean overall firing rate of the cell. I(R|X) is then divided by the mean firing rate of the cell to obtain a spatial information score in bits/spike.

II.6.3.3 Spatial coherence

Spatial coherence is a measure for the 'local smoothness' of the rate map (Muller and Kubie, 1989). It is calculated from unsmoothed rate maps by correlating firing rates in each bin to the mean rate of the 8 surrounding bins. Spatial coherence corresponds to the overall mean of the individual bin correlations. Since spatial coherence is a correlation measure its values were z-transformed for the statistical analysis.

II.6.3.4 Intra-trial correlation of firing rate maps

Intra-trial correlation was obtained by correlating the first half of the trial with the second half by performing a bin-by-bin Pearson's correlation between the two trial halves. For this type of analysis rate maps for both halves of a given trial were adaptively smoothed independently of each other. Unvisited bins in any half of the trial or mutual bins in both halves with a firing rate of 0 Hz were excluded from the analysis. Since intra-trial correlation is a correlation measure its values were z-transformed for the statistical analysis.

II.6.3.5 Inter-trial correlation of firing rate maps

Spatial correlation between two rate maps was obtained by performing a bin-by-bin Pearson's correlation between the respective adaptively smoothed maps. Unvisited bins in any rate map or mutual bins with a firing rate of 0 Hz in both rate maps were excluded from the analysis. Since inter-trial correlation is a correlation measure its values were z-transformed for the statistical analysis.

II.6.3.6 Field analysis

General definition of a firing field

To analyse and define firing fields of place cells the following definitions were applied. The border of a firing field was defined by bins with firing rates lower than or equal to 20% of the overall peak firing rate. A field (main or subfields) had to contain at least 8 contiguous bins. The main firing field of a rate map was the largest of all subfields and furthermore had to also have an in-field peak rate of at least 85% of the overall peak firing rate of the cell. This was done to ensure that on the one hand a small subfield containing a high rate pixel was not deemed the main firing field. On the other hand a large field with a low peak rate will also not be deemed the main field.

All fields (main field and subfields) had to exhibit a mean in-field firing rate of at least the overall mean firing rate of the trial.

Field size

Field size was obtained by calculating the area of the main firing field in cm^2.

Number of subfields

The number of subfields per rate map was obtained by counting the amount of unconnected individual firing fields per rate map (including the main field).

Centre of mass shift

Centre of mass was obtained by finding the location of the weighted centroid of a firing field (weighted by firing rate). Centre of mass shift between rate maps was obtained by calculating the physical distance (in cm) between the centres of mass of two rate maps. Centre of mass always refers to the main firing field.

II.6.4 Single unit classification

II.6.4.1 Clustering of complex spike cells and interneurons

The single unit classification consisted of a two-step process. First, to classify a unit as complex spike cell or interneuron a k-means based clustering algorithm (using the in-built Matlab function 'kmeans') using the squared Euclidean point-to-cluster-centroid distance and assuming two clusters was performed with action potential duration (measured from peak to trough, in ms), the first moment (i.e. mean) of the 20ms-autocorrelation (in ms) and mean firing rate (number of spikes/trial duration, in Hz) as parameters. Autocorrelations were obtained by constructing a frequency distribution of spike trains with a bin size of 0.4 ms across the whole trial. This distribution was used with the in-built Matlab function 'xcorr' (using the 'unbiased' option) to calculate the autocorrelation function across a maximum lag of 20 ms. The first moment corresponds to the mean of this function.

For each unit all parameters were obtained from the first trial where at least 100 spikes were fired. In case a unit never fired 100 action potentials in any trial, the trial with the most spikes was chosen.

All units classified as interneurons were discarded from subsequent data analysis.

II.6.4.2 Place cell definition

Place cells were defined as complex spike cells whose firing exhibited a statistically significant spatial tuning. Place cells were identified by comparing the spatial information content of complex spike cell rate maps to that of a null-population of rate maps based on spike-shuffled data. Spatially shuffled maps were obtained by shifting the spike train of a unit during a given trial by a random amount between a minimum shift of 30 s and a maximum shift of 'trial duration – 30 s'. New spike times that were larger than the trial duration were wrapped around to the beginning of the trial. Shuffled rate maps (adaptively smoothed) were constructed using the shuffled spike train and spatial information content for these shuffled maps was calculated. To obtain a distribution of shuffled spatial information scores, 20,000 spatially shuffled maps for each age bin were constructed. The 95^{th} percentiles of these distributions were used as an age-matched threshold for defining a complex spike cell as place cell (see Figure III-4).

Spatial information was only calculated for shuffled rate maps, where i) number of spikes > 75, ii) path length > 40 m and iii) number of visited bins > 520 (ca. 80% of recording environment). Thus, trials with a bad spatial sampling of the environment and/or cells with low numbers of spikes are excluded.

II.6.4.3 Criteria for inclusion of place cells in analyses

The preceding section described how complex spike cells were defined as place cells (or not) on a single trial. However, all cells in this study were recorded for several trials, possibly including more than one probe trial. A further set of criteria is therefore necessary in order to decide which cells are included into an analysis, depending on the activity of the cell across a given series of trials. The way in which these series of trials were defined, and the criteria used to include place cells in analyses, is described below.

For this thesis, three slightly different inclusion criteria were employed depending on which parts of the experiments (familiar only, probe trial series), and which measures (across-trial vs. within-trial), were to be analysed. Briefly, the different criteria were those applied to i) analysis of place cell properties in the familiar environment (see section II.6.4.3, p. 125); ii) analysis of probe trials for within-trial measures of spatial tuning (see section II.6.4.3, p. 128); iii) analysis of probe trials for across-trial measures of place field stability (see section II.6.4.3, p. 130).

For the analysis of basic place cell properties in the familiar environment only recording trials in this environment before *any* probe trial was run were considered.

For the analysis of probe trials the series of trials used for defining place cells was extended to include the probe itself and any familiar trials preceding or following it. This is because here the properties of place cells are to be compared between 'familiar' and 'probe' trials and place cells that are spatially tuned during the probe but not in the familiar environment (or vice versa) cannot be ignored in the data analysis.

Furthermore, different criteria are applied depending on whether within-trial spatial tuning (see section II.6.4.3, p. 128) or across-trial place field stability (see section II.6.4.3, p. 130) measures are analysed. For the latter case, only cells that are significantly spatially tuned in the

familiar environment (before any probe was run) *and also* exhibit an above threshold average stability in the familiar environment are included. This is because place cells which do not show a spatial tuning or a minimum stability across the familiar trials cannot be meaningfully compared to their respective response in the probe trials (an unstable place cell across familiar trials is not tightly bound to a certain location inside this environment, and thus a possible change of the firing field during the probe cannot be interpreted).

Further detail on each of these three inclusion criteria follows. One general comment has to be made about the potential inflation of type I errors for the method of place cell inclusion. Regardless of the analysis type there are always multiple trials on which a potential place cell could surpass the inclusion criterion, which could result in a multiple comparisons problem. However, since finding the exact absolute percentage of place cells from the whole population at a given age was not an aim of this work, this will not bias the results presented in this thesis. Furthermore, the same method for place cell inclusion was applied to all age bins which would result in a similar number of multiple comparisons for each age bin. This method is much less strict than the criterion used in Wills et al. (2010) where a given unit had to surpass the criterion on every recording trial in an experiment to be deemed a place cell. The number of trials forming an experiment were however much lower in the above mentioned study. Place cell properties in young rat pups can vary quite substantially between recording trials and using a similar criterion for this thesis would generally result in only selecting the best and most reliable place cells in these young animals.

Place cell inclusion for analysis of place cell properties in familiar environment

To define a complex spike cell as place cell for the analysis of basic properties of place cells recorded inside the familiar environment the following criterion was applied. A unit's spatial

information content had to exceed the 95th percentile of the corresponding age-matched distribution of spatial information based on spike-shuffled data in at least one recording trial of a trial series (see Figure II-3). For this type of analysis only recording trials inside the familiar environment before any probe trial was conducted were considered. Recording trials where i) number of spikes < 75, ii) path length < 40 m and iii) number of visited bins < 520 were excluded from this comparison.

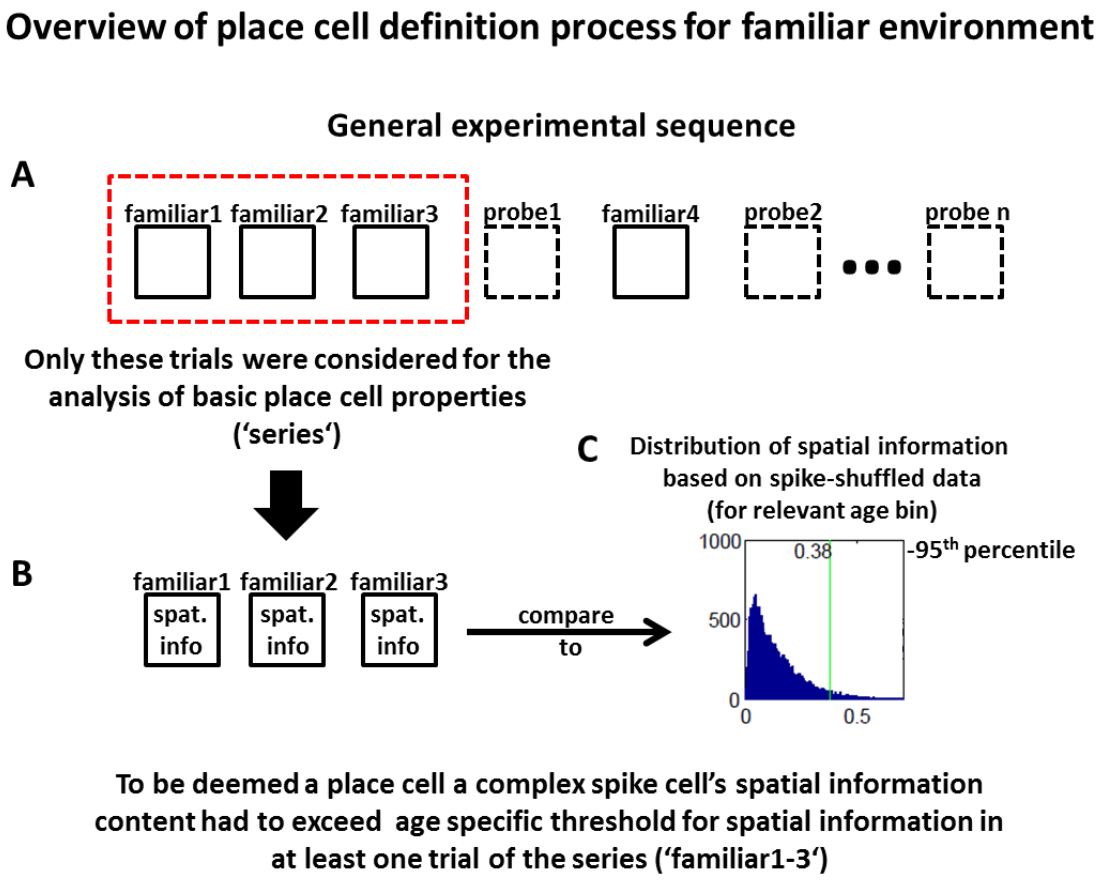


Figure II-3: Schematic overview of place cell inclusion process for the analysis of place cell properties recorded inside the familiar environment before any probe trial was conducted. Top row shows experimental design (A). Red box indicates trials of interest for this type of analysis ('series'). Spatial information content for a given cell was calculated for all trials of a series (B) and these were compared to the 95th percentile of the age-matched distribution of spatial information scores based on spike shuffled data (C). Only cells whose spatial information content exceeded this threshold in at least one trial of the series were deemed place cells.

Calculation of means for place cell properties in familiar environment

Place cell properties in familiar environment

For all included cells, the cell means for this type of data analysis were obtained by averaging the within-trial measures across the recording trials inside the familiar environment before any probe trial was conducted. For the across-trial measures means were obtained by averaging the values of the comparisons 'familiar1' vs. 'familiar2' and 'familiar2' vs. 'familiar3'.

Calculation of 0.05-significance level for basic place cell properties

For calculation of the 0.05-significance level for the age means for spatial information, spatial coherence, intra-trial correlation and inter-trial correlation the following procedure was applied. For each age bin the total number of cells that passed the criterion for the definition as place cells was counted (M). Now M rate maps were selected at random from the population of maps based on spike-shuffled data and the respective place cell properties were averaged across this sample. This procedure was repeated 100,000 times for each age bin producing a distribution of means expected from populations of size M based on spike-shuffled data. The 95^{th} percentile of these distributions was chosen as the 0.05-significance level for the respective place cell property. This means that an average observed value for a given place cell property at a given age exceeding the corresponding age-matched percentile is significantly higher than expected from a size-matched population of units firing without a spatial correlate.

Statistical analysis for place cell properties in familiar environment

For the analysis of the change of place cell properties inside the familiar environment across development, all within- and all across-trial measures were tested separately in MANOVAs with 'age' as the only factor. When a main effect of age was present, Tukey's HSD tests were conducted for the post-hoc analysis.

Place cell inclusion for probe trials: Within-trial measures of spatial tuning

To define a complex spike as place cell for the analysis of the probe trial experiments slightly different criteria were applied depending on which properties of place cells were assessed.

For analysing within-trial properties that characterise the spatial tuning ('spatiality') of place cells (spatial information, spatial coherence, intra-trial correlation, field properties) a similar criterion to that from the analysis of place cell properties in the familiar environment (see Figure II-3) was used, with the exception that the series of trials used for defining place cells was extended to include the probe itself and any familiar environment trials preceding or following it. As previously, any cell whose spatial information content exceeded the 95th percentile of the corresponding age-matched distribution of spatial information based on spike-shuffled data, in at least one recording trial of a trial series, was included in the analysis (see Figure II-3).

For all included cells, the mean values of spatiality in the familiar environment was the mean spatiality across all familiar trials before any probe was run, and the spatiality in the probe was simply the spatiality in that particular probe trial (see Figure II-5C).

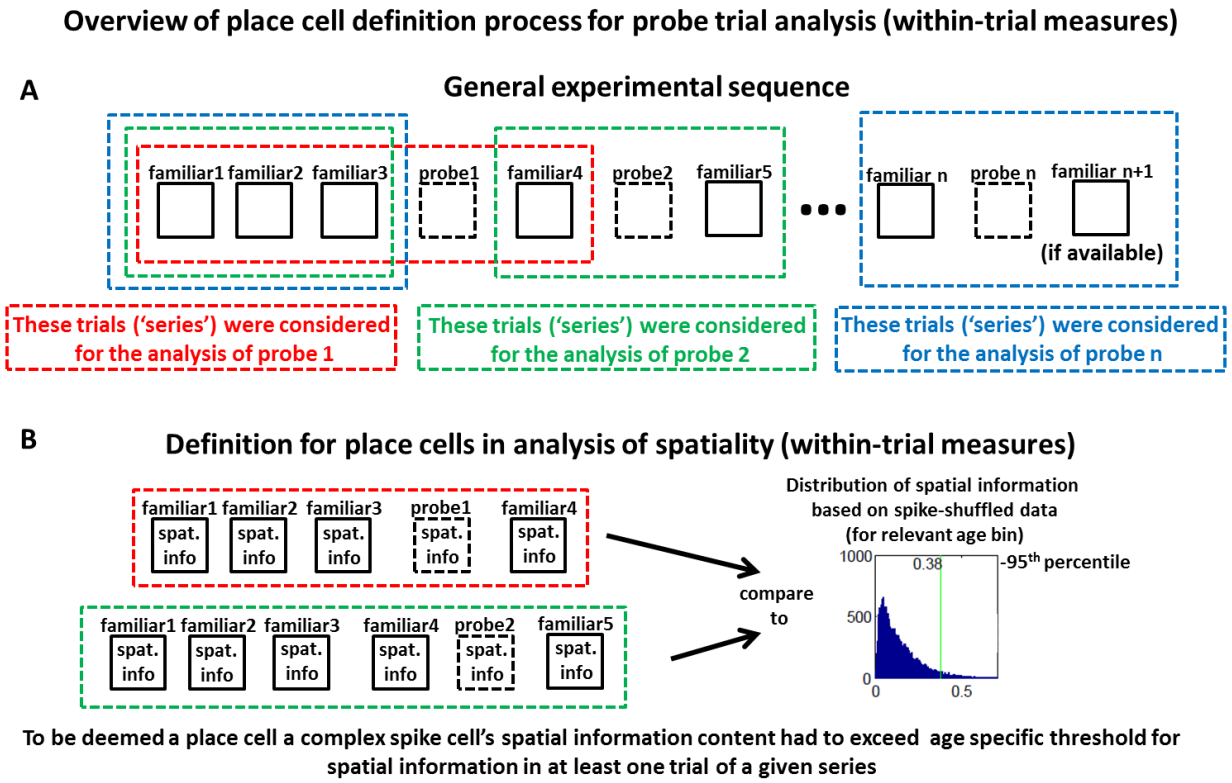


Figure II-4: Overview of method of how place cells were included according to their overall spatiality in a series (within-trial measures). A: general experimental design. B: method of how place cells were defined according to their overall spatiality. Process is shown for two different probe series depending on whether probe of interest was the first (red box) in an experiment or a later one (green box). Note that trials forming the series for a given probe will not include any other probe trials which were part of the experiment. The trials belonging to the series included in the blue box are not shown in B.

Place cell inclusion for probe trials: Across-trial measures of place field stability

For analysing across-trial measures (inter-trial correlation, centre of mass shift) that characterise the stability of the spatial signal of place cells ('stability') a slightly different set of criteria was applied (see Figure II-5A, B). First, a unit's spatial information content for those recording trials inside the familiar environment before any probe trial was conducted had to exceed the 95th percentile of the corresponding age-matched distribution of spatial information based on spike-shuffled data in at least one of those trials. Second, the mean inter-trial correlation across these trials ('familiar1' vs. 'familiar2' and 'familiar2' vs. 'familiar3') had to also exceed the 95th percentile of the corresponding age-matched distribution of inter-trial correlations based on spike-shuffled data (see Figure III-22). These distributions were generated from the same data as for spatial information and constructed using the same methods.

A trial series was defined in the same way as described in the previous section (see section II.6.4.3, p. 128).

Figure II-5C shows how the across-trial comparisons were defined. 'familiar vs. probe' correlation/centre of mass shift corresponds to the inter-trial correlation/centre of mass shift of/between the probe trial and the immediately preceding familiar trial.

Return-to-baseline correlation/centre of mass shift corresponds to the correlation/centre of mass shift between the two familiar trials encompassing a probe trial.

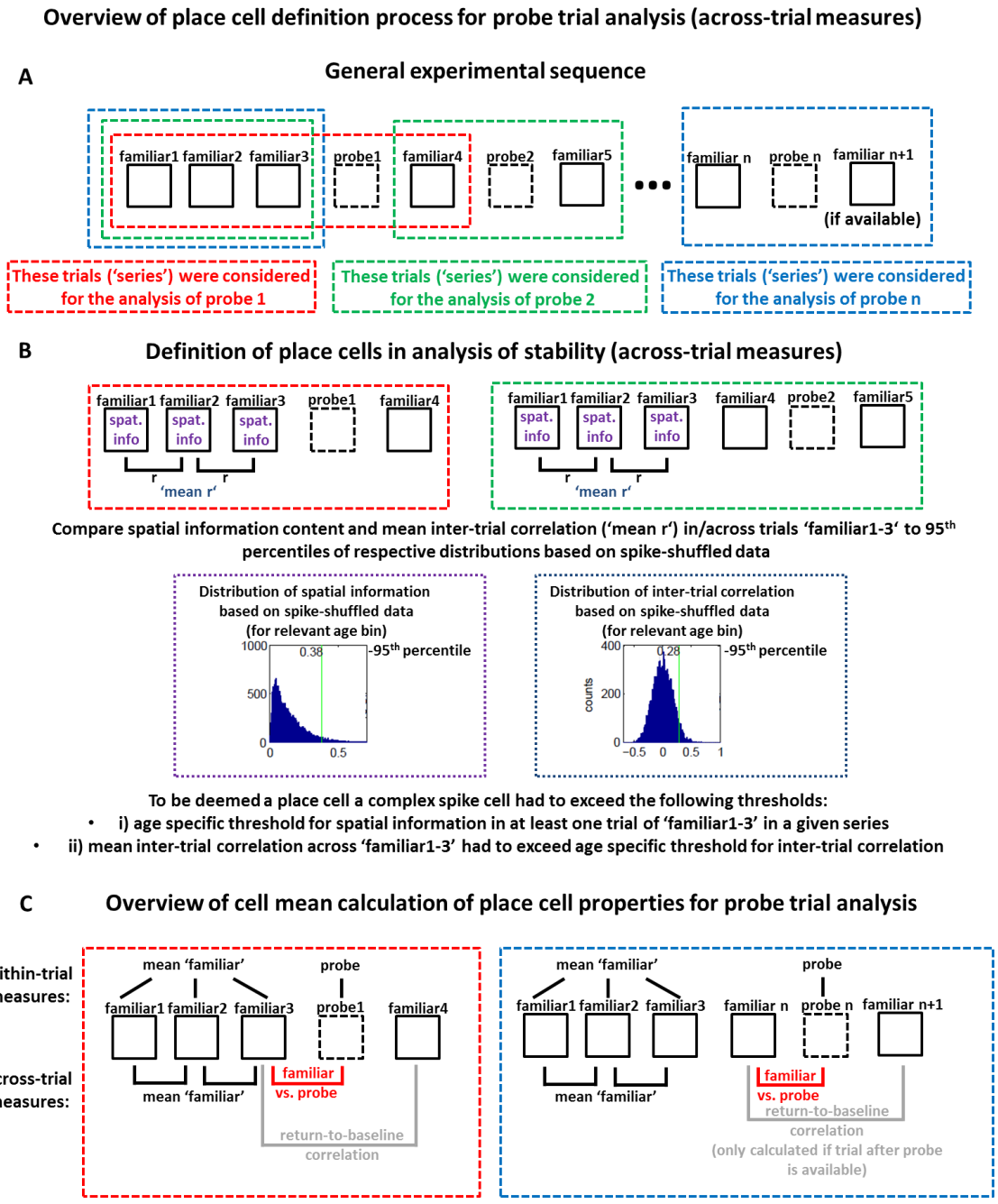


Figure II-5: Overview of method of how place cells were included according to their spatiality and stability in familiar environment. A: general experimental design. B: method of how place cells were defined according to their spatiality and stability across the recording trials inside the familiar environment before any probe was conducted ('familiar1-3'). Process is shown for two different probe series depending on whether probe of interest was the first (red box) in an experiment or a later one (green box). C: Example of how cell means of comparisons were calculated for an individual place cell in a series of trials.

Statistical analysis for probe trial data

The statistical analysis of place cell properties in familiar and probe trials across development consisted of repeated measures ANOVAs for each place cell property in an age-by-environment design. In case a significant interaction between age and environment was present, a simple main effects analysis consisting of the pairwise comparisons at individual age bins was performed to analyse which of the data points actually show a significant difference between familiar and probe trials. When asterisks are indicated in a figure, p-values correspond to the results of these pairwise comparisons.

II.6.5 Calculation of proportions of place cells from complex spike cell population

To calculate the average number of place cells per animal all trials recorded inside the familiar environment before any probe trial was conducted were considered. For each animal the proportion of place cells was calculated. These proportions were averaged across the respective age bins. To be able to better compare the proportions to the results from Wills et al. (2010) the same method of calculation from that study was included in addition. The method used in this thesis defined a place cell across a series of trials, while in Wills et al. (2010) place cells were defined on a single trial basis. In brief, if a given unit exceeded the age-specific threshold in one out of three trials of a trial series, it was deemed a place cell after the method employed in this thesis, while with the method of Wills et al. (2010) this would return a proportion of 33%.

Calculation of 0.05-significance level for proportions of place cells

To calculate the 0.05-significance level of defining a unit as place cell by chance from a shuffled rate map, the following calculation was employed. For each age bin the total number of cells (method in this study) or rate maps (method in Wills et al. (2010)) that passed the criterion was counted (M). M rate maps were selected at random from the population of maps based on spike-shuffled data and the proportions of those passing the criterion were calculated. This procedure was repeated 100,000 times to obtain a distribution of means expected from populations of size M based on spike-shuffled data. The 95th percentile of these distributions was chosen as 0.05-significance level for the proportions of cells per animal for each age bin.

II.6.6 Field-to-wall distance vs. place cell stability

For the correlation between field-to-wall distance and place cell stability (inter-trial correlation) both measures were averaged for each place cell across trials inside the familiar environment before any probe trial was conducted. Field-to-wall distance corresponded to the distance of the weighted centre of mass of the main firing field of a place cell to the nearest environmental boundary.

For subsampling the correlations for age bins P14-15, P16-17, P18-19, P20-21 and adult controls by the number of place cells included in this type of analysis for P22-27 (C), the following procedure was employed. For each age bin C data points were selected at random and the correlation between field-to-wall distance and place cell stability was assessed. This

procedure was repeated 10,000 times for each age bin to yield a distribution of r values based on the subsampled population.

II.6.7 Active vs. inactive cells during probe trials

To assess the number of cells that either showed exclusive activity during the probes ('probe only') or probe-specific ceasing of activity ('familiar only') the following criteria were applied: To be classified as 'probe only' a unit had to fire > 75 spikes during the probe trial and < 75 spikes during all familiar trials of the probe specific trial series. To be classified as 'familiar only' a place cell had to fire < 75 spikes during the probe trial and > 75 spikes in the familiar trials encompassing the probe trial. This means that units which e.g. were active in the familiar trials before the probe was run, then ceased firing during the probe, but also remained silent in the following familiar trial were not deemed as 'familiar only'. In case a probe trial was the last trial in a series, a unit had to be active in at least one of the familiar trials before the probe was run and during the familiar trial immediately preceding the probe trial. To be classified as active in both environments, a cell had to fire > 75 spikes in any familiar trial of a series as well as in the probe trial. Note that occasionally cells cannot be conclusively assigned to any of those categories, due to an inconsistent firing behaviour across the familiar trials (e.g. a cell that fired in only one or two familiar trial in the series whilst being silent in all other familiar trials).

II.6.8 'Knock-on' analysis

To analyse possible 'knock-on' effects of probe trials (changes to place cell firing during the following familiar trials), place cells were classified as different cell types according to the following criteria:

Type 1=all stable: Stable place field throughout probe exposure and following familiar trial, (return-to-baseline correlation and familiar vs. probe correlation > threshold).

Type 2=probe only remapping: Change in field position in response to probe, and return to 'correct' firing in following familiar trial (return-to-baseline correlation > threshold and familiar vs. probe correlation < threshold)

Type 3=r2b only remapping: No change in field position in response to probe, but field position changed during following familiar trials (return-to-baseline correlation < threshold and familiar vs. probe correlation > threshold)

Type 4=all remapping: Change in field position in response to probe, but field position also changed during following familiar trials (return-to-baseline correlation and familiar vs. probe correlation < threshold)

Type 5=not classifiable: Other; i.e. cells that were not active in any one of the trials of interest (familiar trial before probe, probe trial or familiar trial after probe).

The threshold was the respective 95^{th} percentile of the distribution of inter-trial correlations based on spike-shuffled data (see Figure III-22). Only those probe trials which were encompassed by two trials inside the familiar environment were included in this analysis. Units which ceased firing during the probe trial or one of the familiar trials were not included in this analysis (type 5=not classifiable). Data from all probes was pooled for the 'knock-on' effects

analysis. For the calculation of the percentages of each cell type for the different age bins, all units which for any experiment were classified as type 3=r2b only remapping or type 4=all remapping were deemed to show a 'knock-on' effect. For the classification of showing no 'knock-on' effect a cell had to be classified as type 1=all stable or type 2=probe only remapping for all probes run with this cell. This was done to avoid double counting, especially for adult data as here usually all (or at least more than two) probes were run in one single experiment. For the analysis of 'knock-on' effects the place cell population defined by their spatiality and stability across familiar trials before any probe was run was used.

II.7 Histology

II.7.1 Rat pups

At the end of an experiment rat pups were killed using an overdose of sodium pentobarbital (Euthatal; Merial Animal Health; Harlow, UK) and perfused transcardially with physiological saline (1-2 min) followed by 4%-paraformaldehyde (PFA; VWR; Leicestershire, UK) (5-8 min). Brains were dissected and kept in 4%-PFA until processing. 24 hours prior to sectioning, brains were transferred to 4%-PFA-30%-sucrose for cryoprotection. Brains were then cut coronally in 30 µm sections with a Cryostat (model OTF, Bright Instruments; Huntingdon, UK) and mounted on microscopic slides (superfrost; BDH; Bristol, UK). Slides were air-dried overnight. Sections were stained with 0.1%-Cresyl-violet (Nissl staining, Sigma-Aldrich®; Gillingham, UK) using a standard protocol. After the staining procedure, slides were immediately cover-slipped with DPX (Sigma-Aldrich®; Gillingham, UK).

II.7.2 Adult rats

Adult rats were perfused in a similar way except for slightly longer times for saline (2-3 min) and PFA (ca. 10 min) perfusion. The processing of brain tissue for histology was done in the same way as for rat pups.

II.7.3 Microscopy

To confirm electrode locations, images were taken from the brain slice with the deepest point of the electrode track with a microscope (Leica DM750) at a magnification of 2.5x. A 10% shrinkage correction was applied on the images to account for tissue shrinkage.

III Results

III.1 Introduction to results section

III.1.1 General remarks

The following chapter will describe the results of the experiments undertaken for this thesis. It is split up into six subsections. The first part will describe in detail how single unit data was processed to differentiate between complex spike cells and interneurons as well as how place cells were defined (see section III.2). The next subsection will give a description of the properties of place cells when recorded in a familiar environment (see section III.3). This is followed by a characterisation of the responses of place cells when probed with certain manipulations of the familiar environment as well as recordings in a completely novel environment (see section III.4). During these probe trials certain aspects of sensory stimuli within the familiar environment were manipulated and the responses of place cells are then compared to the spatial representation of the familiar environment. Note that the unit of analysis will consist of single cells and not animals. This is due to practical reasons since unlike in adult animals where large cell populations can be recorded on a regular basis in single animals, it is much more difficult to obtain these cell numbers in recordings from developing rats.

III.1.2 Selection of age bins

The following age bins were selected to assess developmental changes of place cell properties and functioning: P14-15, P16-17, P18-19, P20-21, P22-27 and adult controls. The main focus of this thesis lies on the developmental changes prior to weaning (i.e. until P21) which is why all the data from post-weanling pups were pooled in one single age bin (P22-27). Furthermore, as described in the introduction, the sensory development until P21 includes the anatomical and functional emergence of all sensory modalities, i.e. olfaction, hearing, touch and vision (see section I.4.3). Moreover, P21 also denotes the functional emergence of grid cells (Wills et al., 2010) and thus all younger ages should represent times where the functioning of place cells will be observed without functional input from grid cells in MEC.

III.1.3 Animal and cell numbers

For this thesis, recordings from the hippocampus from a total of 27 rat pups aged P14-27 and 10 adult control animals were undertaken. Recordings from rat pups were obtained from CA3 as well as different anterior-posterior levels of CA1. Recordings from adult controls were always conducted from posterior locations in CA1. In total, 1432 single units were recorded across all experiments and animals. 1196 single units were recorded from rat pups and 236 from adult controls.

III.2 Single unit and place cell classification

III.2.1 Separation of complex spike cells and interneurons

III.2.1.1 Spiking properties separating complex spike cell and interneuron population

As described in the introduction of this thesis, the hippocampus of rodents contains two broad groups of cell types: Pyramidal cells (complex spike cells) and interneurons. Thus, the first step of the data analysis was to separate these cell types based on their firing properties (see section I.2.4). While pyramidal cells exhibit so-called complex spiking (Fox and Ranck, 1975; Ranck, 1973), i.e. bursts of 2-6 action potentials, broader waveforms and generally fire at lower average rates, interneurons show quite different firing properties. The action potentials of this cell group typically have a narrower waveform, cells fire at higher average rates and do not show bursts of action potentials, but a rather regular sustained activity. For this thesis i) the width of the waveforms, measured from peak to trough, ii) the first moment (i.e. the mean) of the autocorrelation (across 20 ms) and iii) the mean firing rate were chosen as parameters to distinguish between pyramidal cells and interneurons.

The first moment essentially is a good measure to distinguish 'bursty' from 'non-bursty' neurons, as the former will have a shorter first moment than the latter. This is because a burst of action potentials will invariably move the mean of the autocorrelation to shorter time points. Note that because almost all units (complex spike cells as well as interneurons) in the hippocampus show some form of theta modulation (at ca. 4-12 Hz), the 20ms-autocorrelation will only contain the first theta peak. Figure III-1 gives some representative examples (spikes

whose parameters fall within the range of respective age mean±standard deviation for all measures) on how these measures were obtained (spike width: A1 and B1, first moment of autocorrelation: A2 and B2, mean rate: A3 and B3). The units shown in Figure III-1 exemplify the difference in spike duration, the first moment of the autocorrelation and mean firing rate between complex spike cells and interneurons. The 20ms-autocorrelations highlight the 'burstiness' of the former and the rather regular activity of the latter, since only complex spikes show a clear well separated peak in the autocorrelation (see Figure III-1, leftmost panels in A2 and B2). In contrast, interneurons do not show a clear peak but a step-like (adult example) or more gradual (pups example) increase in the likelihood of firing an action potential with increasing time lags. Taken together all these spiking properties are known to reliably separate the two main groups of cell types in the hippocampus of adult rats (Csicsvari et al., 1999).

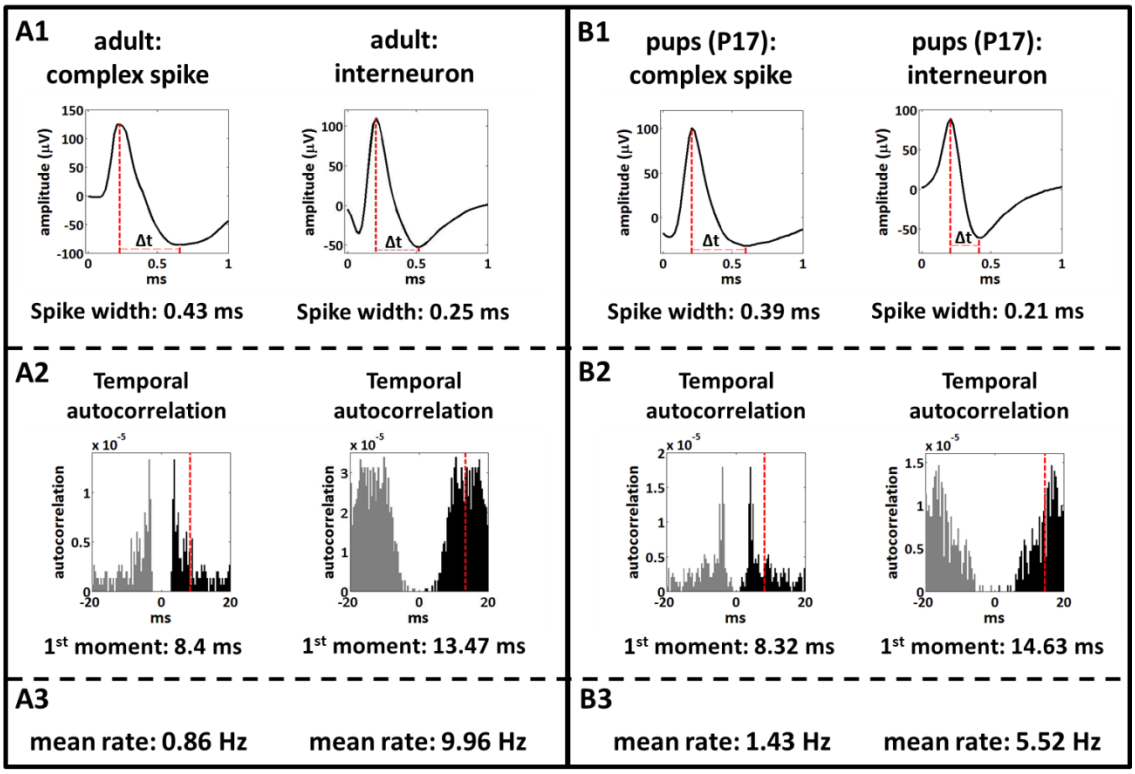


Figure III-1: Representative examples of units exemplifying how spike duration and first moment of the autocorrelation were obtained. A1-3: Units recorded from adult controls (left: complex spike cell, right: interneuron). B1-3: Units recorded from rat pups (left: complex spike cell, right: interneuron). Top panels show mean waveforms of units from the channel with the highest signal amplitude. Δt corresponds to spike duration, measured from peak to trough (red dashed lines indicate maximum and minimum of waveform). Panels in middle show 20ms-autocorrelations. Red dashed line corresponds to first moment. Bin size for the autocorrelations is 0.4 ms. Central peak is not shown. Bottom panel indicates mean firing rates (Hz). All examples are actual recorded units and have spiking properties (spike width, first moment of autocorrelation and mean rate) in the range of age mean±standard deviation. ms: milliseconds, µV: micro Volt, Hz: Hertz

III.2.1.2 k-means based clustering of single units according to spiking properties

Figure III-2 shows the result of a k-means based clustering (using the squared Euclidean distance of the point-to-cluster-centroid distances) over the three measures (spike width, first moment of autocorrelation and mean firing rate), assuming two clusters in total. For the adult dataset this includes 183 units classified as complex spikes and 53 as interneurons. For the pups dataset these are 1047 and 149 units, respectively. The scatterplots show that the clustering overall separates the two cell types fairly well across the relevant properties (see Figure III-2). Nearly all units with high mean firing rates (> 5-10 Hz) are assigned to the interneuron cluster (red dots) while units with a broader average waveform (> 0.4 ms) are almost exclusively found in the complex spikes cluster (blue dots). It seems however that the clustering is somewhat better for units recorded from adult animals (see Figure III-2B) compared to rat pups (see Figure III-2C), as for the former the two clusters seem to be better separated. This seems to be due to the slightly longer spike durations of interneurons in the rat pup dataset, as e.g. in the adult dataset there are only two units exceeding a spike width of 0.3 ms. In the pups dataset there are several units assigned to the interneuron cluster with a spike duration of ca. 0.3-0.4 ms. Also, the pup dataset hardly contains any units with firing rates exceeding 20 Hz (complex spikes or interneurons). However, further support for the reliability of the cluster separation can be derived from the means of each cluster for relevant measures that characterise the spiking properties in more detail (see Figure III-3 and Table III-2).

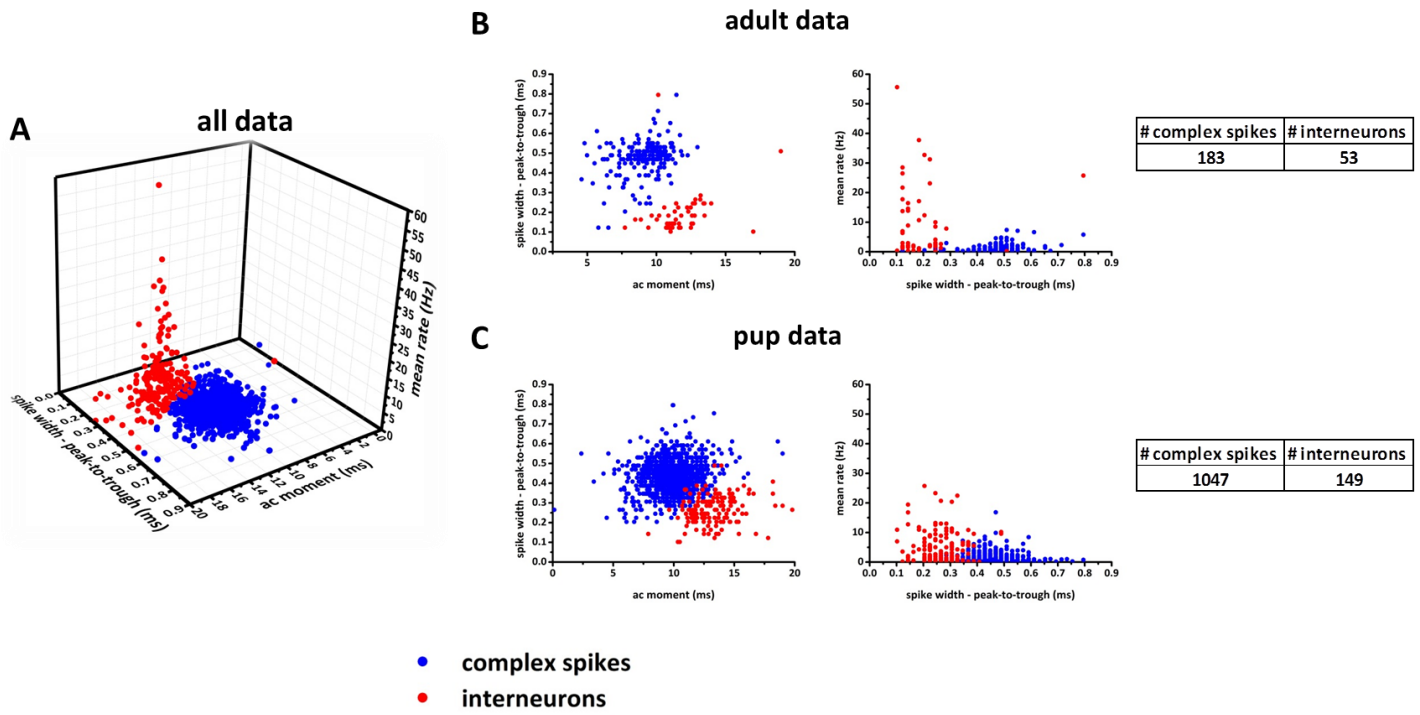


Figure III-2: Scatterplots showing the result of the k-means based clustering. A: 3-D scatterplot (across spike width (ms), first moment of autocorrelation (ms) and mean firing rate (Hz)) for all data pooled together. B and C show 2-D scatterplots of the same data separated between adult controls (B) and rat pups (C). Red dots correspond to interneurons, blue dots to complex spike cells. Tables show numbers of complex spike cells and interneurons for adult (top) and pup (bottom) datasets. ac: autocorrelation, ms: milliseconds, Hz: Hertz.

III.2.1.3 Proportions of complex spike cells and interneurons from the recorded cell population

The results of the first single unit classification step can be summarised as follows (see Table III-1). From a total of 1432 units recorded in this study 1230 (86%) were classified as complex spikes cells and 202 as interneurons (14%). From the total number of 1432, 1196 units were recorded from rat pups aged P27 or younger, of which 1047 (88%) were classified as complex spike cells. The remaining 236 units from the total of 1432 were recorded from adult control animals, of which 183 (78%) were classified as complex spike cells.

Table III-1 shows a summary of the proportions of complex spike cells for all age bins from the recorded cell population. Note that in adult controls the percentage of complex spike cells is about 10% lower than for any of the age bins containing rat pup data, except for P16-17 (6% lower) and P22-27 (16% lower). All age bins including data from rat pups show fairly similar proportions of complex spikes (ca. 85-95%).

Table III-1: Overview of numbers of recorded complex spike cells and interneurons. Table shows cell numbers for all data pooled together as well as a separation of adult and rat pup data. Cell numbers for all individual age bins containing data recorded from rat pups are shown as well.

	# all cells	# complex spikes	# interneurons	% complex spikes
all data	1432	1230	202	86
adults	236	183	53	78
pups	1196	1047	149	88
P14-15	142	127	15	89
P16-17	329	277	52	84
P18-19	361	314	47	87
P20-21	268	239	29	89
P22-27	96	90	6	94

III.2.1.4 Physiological properties of complex spike cells and interneurons

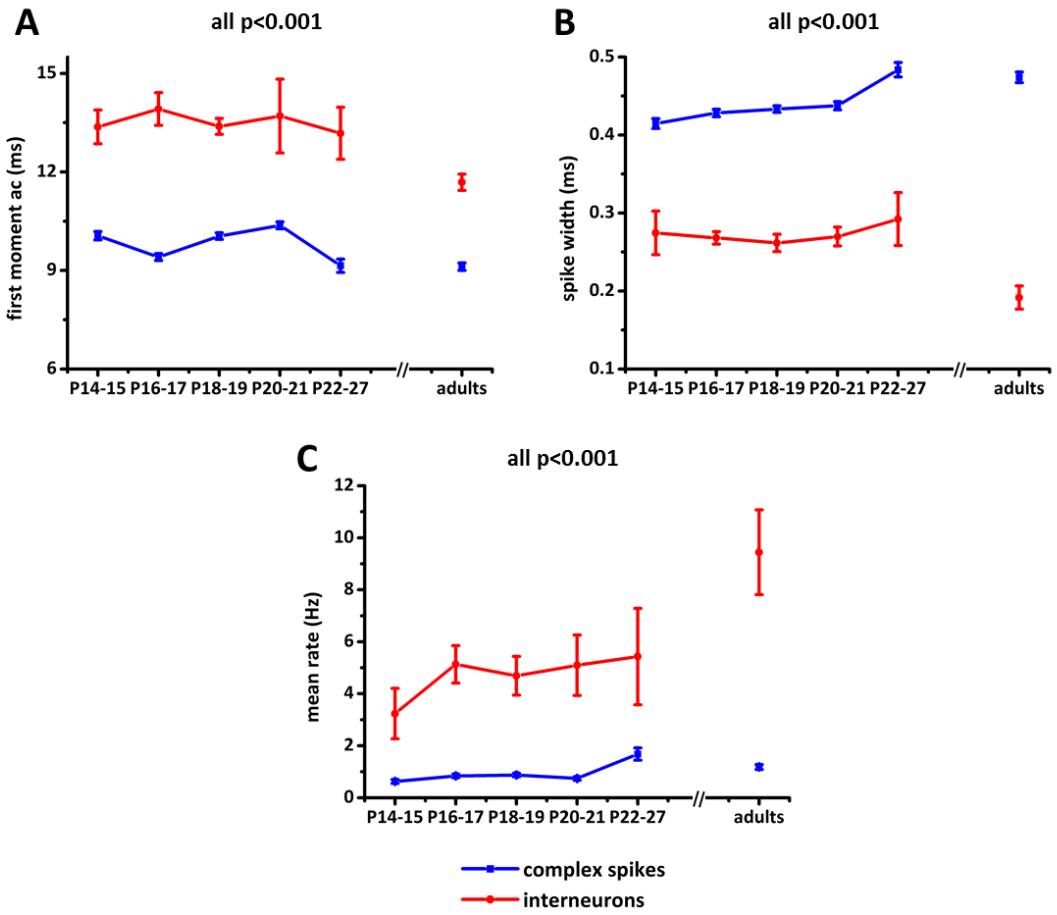


Figure III-3: Comparison of physiological properties of complex spike cells and interneurons across development. Depicted are first moment of the 20ms-autocorrelation function (A), spike width measured from peak to trough (B) and mean firing rate (C). Blue lines correspond to complex spike cells and red lines to interneurons. For all age bins values differ significantly between the two cell types (all p<0.001). All values are means±SEM. ms: milliseconds, ac: autocorrelation, Hz: Hertz

Table III-2: Means of spiking properties of complex spike cells (top half) and interneurons (bottom half). Shown are first moment of the 20ms-autocorrelation (in ms), spike width measured from peak to trough (in ms) and mean firing rate (in Hz). For all measures the standard deviation (std) is shown as well. The respective means are given for the whole pooled dataset (all data), adult controls (adults), and rat pup data pooled together (pups). ac: autocorrelation

complex spike cells	cell numbers	ac moment	±std	spike width	±std	mean rate	±std
all data	1230	9.76	1.76	0.44	0.08	0.92	1.24
adults	183	9.11	1.56	0.47	0.09	1.18	1.28
pups	1047	9.88	1.76	0.44	0.08	0.87	1.22
interneurons	**cell numbers**	**ac moment**	**±std**	**spike width**	**±std**	**mean rate**	**±std**
all data	202	13.12	3.38	0.25	0.09	6.01	7.79
adults	53	11.69	1.81	0.19	0.11	9.44	11.85
pups	149	13.63	3.65	0.27	0.07	4.81	5.27

Figure III-3 and Table III-2 give an overview of the means of the physiological properties (spike width, first moment of autocorrelation and mean firing rate) of complex spike cells and interneurons after assigning recorded units to the two groups based on a k-means clustering algorithm (see Figure III-2). Figure III-3 shows a comparison of properties across development and Table III-2 indicates the means for adults and all rat pup data pooled together.

To understand whether these properties (spike width, firing rate and first moment of autocorrelation) differ between the two cell types in general and also at each individual age bin an omnibus Multivariate Analysis of Variance (MANOVA) with a 3x2x6 design (3 dependent variables across cell type and age) was performed. This analysis reveals a main effect of cell type ($F_{3,1418}$=481.43, p<0.001), a main effect of age ($F_{15,4260}$=9.27, p<0.001) as well as a significant interaction between cell type and age ($F_{15,4260}$=9.86, p<0.001). The between-subjects effects for cell type highlight the different properties of interneurons and complex spikes. Just

as expected the complex spike cell cluster has a broader average waveform (all data: complex spike cells: 0.44±0.08 ms vs. interneurons: 0.25±0.09 ms; $F_{1,1420}$=526.21, p<0.001), a lower average firing rate (all data: complex spike cells: 0.92±1.24 Hz vs. interneurons: 6.01±7.79 Hz; $F_{1,1420}$=222.38, p<0.001) as well as a smaller first moment of the autocorrelation (all data: complex spike cells: 9.76±1.76 ms vs. interneurons: 13.12±3.38 ms; $F_{1,1420}$=359.42, p<0.001). The difference between complex spike cells and interneurons for the latter measure stresses the different bursting properties of the two cell groups. All three properties differ significantly between complex spikes and interneurons for all age bins (pairwise comparisons; all p<0.001).

Interestingly, there also seems to be a developmental change of all three properties as the interaction parameter between cell type and age is significant for all measures (first moment: $F_{5,1420}$=5.42, p<0.001; spike width: $F_{5,1420}$=13.3, p<0.001; mean rate: $F_{5,1420}$=12.97, p<0.001). Table III-3 gives an overview of all post-hoc pairwise comparisons of all parameters across the different age bins for complex spike cells and interneurons.

For complex spike cells there is steady increase in spike width between P14-15 and P22-27 (P14-15: 0.41±0.07 ms; P22-27: 0.48±0.09 ms; adults: 0.47±0.09 ms) at which point the adult level is reached (see Table III-3). This is accompanied by a decrease in the first moment of the 20ms-autocorrelation (P14-15: 10.06±1.44 ms; P22-27: 9.14±1.93 ms; adults: 9.11±1.56 ms), which also shows a rather gradual change if data recorded at P16-17 is neglected. Again adult levels are reached for the first moment at P22-27 (see Table III-3). Complex spike cells also show a moderate increase in mean firing rate (P14-15: 0.63±0.83 Hz; P22-27: 1.68±2.26 Hz; adults: 1.18±1.28 Hz) across development. However, only data recorded at P22-27 reveals significantly higher average firing rates than any other group. All other age bins do not differ in their average firing rates (see Table III-3).

For interneurons some developmental changes are present as well, in that adult values differ from all pup values (see Table III-3). Spike width does not change significantly across the age

bins containing rat pup data, but must decrease at a later time point in development as all these age bins show significantly higher spike durations compared to adult controls (pups: 0.27±0.07 ms; adults: 0.19±0.11 ms). The change of the first moment of the 20ms-autocorrelation (pups: 13.63±3.65 ms; adults: 11.69±1.81 ms) and mean firing rate (pups: 4.81±5.27 Hz; adults: 9.44±11.85 Hz) across development shows similar results with the exceptions of the comparison between P16-17 and P22-27 for the first moment and the comparisons between P14-15 and P16-17 as well as P14-15 and P20-21 for mean firing rate (see Table III-3).

These are quite interesting results because they point out to different developmental trajectories for the spiking properties of the two cell types. On the one hand, complex spikes show a more gradual development (except for mean rate) in their spiking properties and adult levels are reached for all parameters in post-weanling pups. On the other hand, interneurons show no developmental change between P14-27 in their spiking properties.

Table III-3: Overview of post-hoc comparisons from the statistical analysis of spiking properties. Left half shows results of comparisons for complex spike cells and right half those for interneurons. Red values indicate significant p-values (below 0.05-level).

Complex Spikes							Interneurons						
spike width	P14-15	P16-17	P18-19	P20-21	P22-27	adults	spike width	P14-15	P16-17	P18-19	P20-21	P22-27	adults
P14-15		0.12	0.03	0.01	<0.001	<0.001	P14-15		0.73	0.59	0.71	0.65	<0.001
P16-17			0.45	0.2	<0.001	<0.001	P16-17			0.78	0.95	0.46	<0.001
P18-19				0.55	<0.001	<0.001	P18-19				0.86	0.38	<0.001
P20-21					<0.001	<0.001	P20-21					0.46	<0.001
P22-27						0.36	P22-27						0.004
first moment	P14-15	P16-17	P18-19	P20-21	P22-27	adults	first moment	P14-15	P16-17	P18-19	P20-21	P22-27	adults
P14-15		<0.001	0.95	0.09	<0.001	<0.001	P14-15		0.79	0.97	0.17	0.82	0.001
P16-17			<0.001	<0.001	0.21	0.07	P16-17			0.73	0.03	0.66	<0.001
P18-19				0.03	<0.001	<0.001	P18-19				0.06	0.78	<0.001
P20-21					<0.001	<0.001	P20-21					0.47	0.02
P22-27						0.89	P22-27						0.04
mean rate	P14-15	P16-17	P18-19	P20-21	P22-27	adults	mean rate	P14-15	P16-17	P18-19	P20-21	P22-27	adults
P14-15		0.52	0.44	0.73	0.01	0.12	P14-15		0.03	0.11	0.04	0.14	<0.001
P16-17			0.89	0.73	0.02	0.24	P16-17			0.38	0.95	0.88	<0.001
P18-19				0.62	0.03	0.28	P18-19				0.42	0.57	<0.001
P20-21					0.01	0.14	P20-21					0.91	<0.001
P22-27						0.21	P22-27						0.002

III.2.2 Complex spike cells and place cells

III.2.2.1 Place cell characterisation

Because not all complex spike cells can be considered to be place cells (O'Keefe, 1979) and the clustering of the data according to spiking properties is not perfect (see Figure III-2), a second filtering step was employed to only select those complex spike units that exhibit a significant spatially tuned response during the exploration of the experimental environment. Spatial information (Skaggs et al., 1993) is a measure to characterise the quality of the spatial tuning of a given place cell, and was thus chosen as a criterion to define place cells. In this thesis a complex spike cell was deemed a place cell when its spatial information exceeded a certain threshold in at least one trial of a given trial series (see Figure III-12 for recordings in familiar environment only, and Figure III-21 for probe trial analysis for an explanation of what is a trial series).

The threshold values are computed as follows: The spike trains of all recorded complex spike units (as defined by the k-means based clustering) were shuffled by shifting respective spike times by the same pseudo-randomly chosen amount. Rate maps were constructed using the temporally shuffled spike trains and spatial information scores for these shuffled rate maps were calculated (see Figure III-4A). This was repeated until there were 20,000 shuffled rate maps for each age bin, yielding an age-matched distribution of spatial information scores based on spike-shuffled data (see Figure III-4B). The 95^{th} percentiles of these distributions were used as a threshold for characterising a complex spike cell as place cell. It is important to employ age specific thresholds since the quality of the spatial signal is known to improve with age (Langston et al., 2010; Martin and Berthoz, 2002; Scott et al., 2011; Wills et al., 2010) and

because spatial information values could be biased, e.g. by general changes in firing rates across age.

Figure III-4B shows the distribution of spatial information scores obtained from shuffled rate maps for each respective age bin. The green line depicts the 95th percentile of each distribution and only cells that passed this criterion in at least one of the recording trials of a given series were considered to be genuine place cells. All histograms look fairly similar across all age bins, as they show a skewed distribution towards zero. The main difference between age groups is the size of the tail containing the higher values (> 0.3 bits/spike), which also explains the slight differences in the absolute value of the 95th percentile across different age bins.

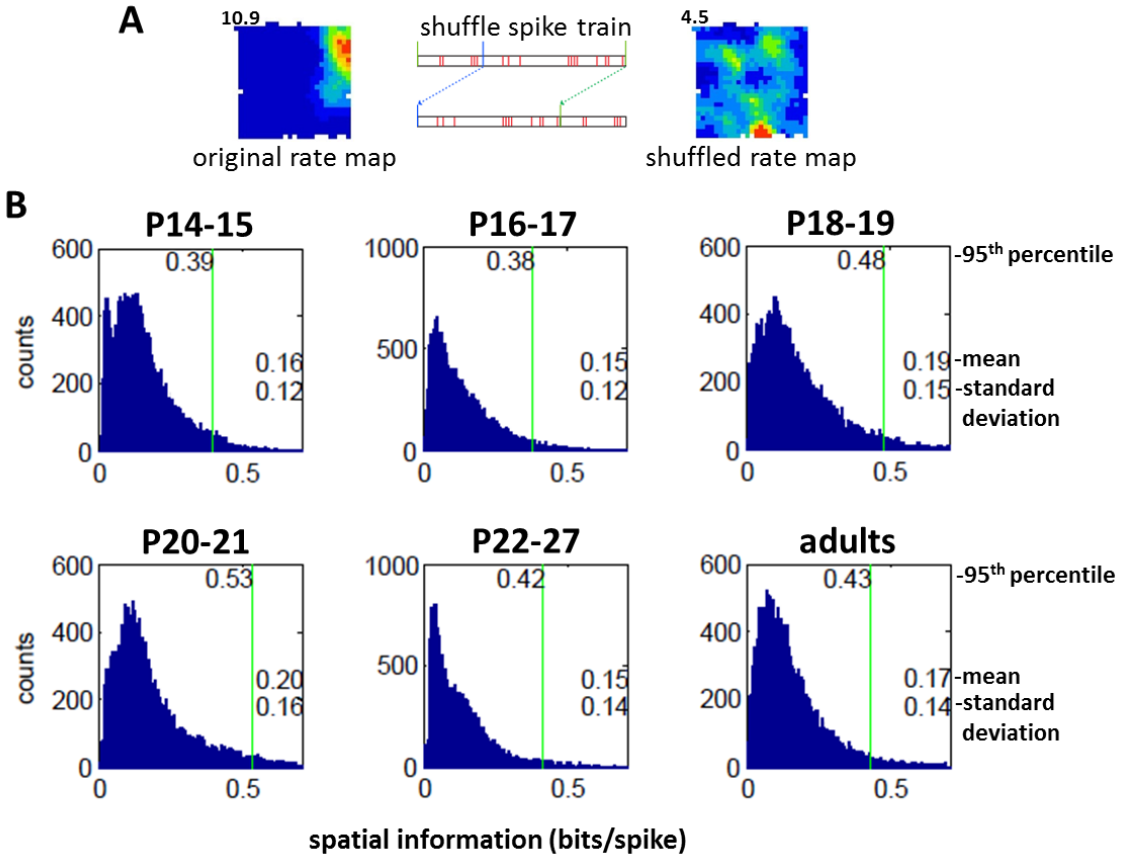


Figure III-4: Overview of method of how cells were classified as place cells. A: Schematic of shuffling procedure with actual rate map on the left and its shuffled counterpart on the right. B: Histograms show distributions of spatial information values of shuffled rate maps (20,000 samples per histogram) for all age bins (from top left to bottom right in B). Green lines depict 95th percentile of distributions (numeric value is indicated at top left to green line) and values in middle right of each histogram show mean (upper value) and standard deviation (lower value) of distributions.

III.2.2.2 Proportion of place cells from complex spike cell population

By classifying place cells based on their spatial information scores exceeding a certain age-dependent threshold, the following proportions of the complex spike cell population were deemed place cells (see Table III-4). Note that these values only refer to the recording trials

undertaken in the familiar environment before any probe trial was conducted. Numbers of place cells recorded for the individual probe trials will be presented in the respective sections.

This filtering algorithm classified 519 out of the total of 1108 complex spike cells recorded inside the familiar environment as place cells (47%). This percentage was much higher for units recorded from adult control animals, where ca. 81% (116/144) of the recorded complex spike cells were classified as place cells. For rat pup data the overall percentage of place cells from the complex spike cell population was 42% (403/964), which is also very close to the respective proportions of the individual age bins containing rat pup data.

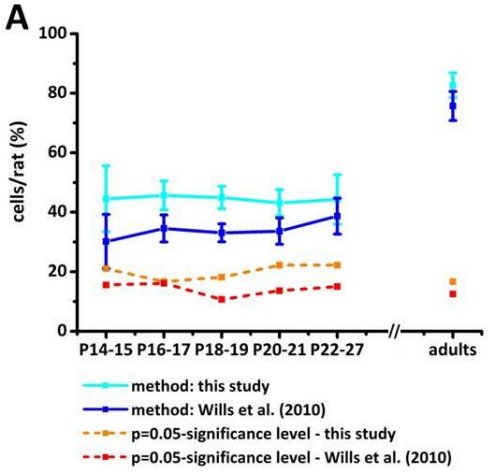
Table III-4: Proportion of place cells from complex spike cell population recorded in familiar environment across all age bins. Shown is the result of the filtering algorithm based on spatial information. Note that the numbers are only referring to recordings inside the familiar environment before any probe trial was conducted.

	# complex spikes	# place cells	% place cells
all data	1108	519	47
adult	144	116	81
pups	964	403	42
P14-15	114	49	42
P16-17	252	108	43
P18-19	295	121	41
P20-21	221	91	41
P22-27	82	34	42

The proportion of complex spike cells which are place cells does not appear to change between P14 and P27, in contrast to a previous study (Wills et al., 2010), where this proportion was found to increase gradually over a similar age range. It should be noted that the table above shows the percentage of all cells pooled across rats, whereas Wills et al. (2010) reported

the mean proportion of place cells per rat. However, even when the proportions of place cells are calculated per animal, similar results are obtained (see Table III-4 and Figure III-5A). Furthermore, if exactly the same method as in Wills et al. (2010) is replicated (see section II.6.5), the proportion of place cells per animal is substantially lower (main effect of method: $F_{1,168}$=8.59, p=0.004), but the trend over the ages P14-27 remains flat. We can therefore conclude that the proportion of complex spike cells showing significant spatial tuning in this study does not replicate the results shown in at least one other previous work. Note that for both methods the proportions of place cells from the complex spike cell population do not increase with age for data from rat pups, and that the adult proportions are much higher than in rat pups (main effect of age: $F_{5,168}$=15.38, p<0.001; see Figure III-5B for post-hoc statistical analysis). The interaction between age and method is non-significant ($F_{5,168}$=0.12, p=0.99). Post-hoc tests for age show that all cell proportions in rat pups differ significantly from the ones in adult controls, but not amongst each other (see Figure III-5B). Both methods yield significantly higher proportions of place cells than expected from chance (compare cyan and blue line against red and orange dashed lines in Figure III-5A).

A



B

Cells per Rat	P14-15	P16-17	P18-19	P20-21	P22-27	adults
P14-15		1	1	1	0.99	<0.001
P16-17			1	1	1	<0.001
P18-19				1	1	<0.001
P20-21					0.99	<0.001
P22-27						<0.001

Figure III-5: Proportions of place cells per animal that pass the criterion for a significant spatial tuning across development. Proportion is shown after calculation with two different methods (A). Cyan line shows place cell proportions using definition employed in this thesis and dark blue line shows proportions calculated after Wills at al. (2010). Red and orange dashed lines indicate 0.05-significance level for mean proportion expected from spike shuffled data for both methods, respectively. B gives overview of results of the post-hoc analysis for age. P-values are indicated for all comparisons. Red values indicate significant results (0.05-significance level).

III.2.2.3 Quality of spike sorting of place cells

To exclude that any possible developmental effects in place cell functioning are due to differences in the quality of the single unit clusters obtained through manual spike sorting (see section II.6.1) between different ages, two measures that characterise this quality were calculated and compared across age bins (see Figure III-6). These are isolation distance (Harris et al., 2001) and L-ratio (Schmitzer-Torbert and Redish, 2004), which are both known to reliably characterise the cluster quality of hippocampal recordings (Schmitzer-Torbert et al., 2005). In brief, the former describes the distance of the cluster spikes to all other spikes recorded on the same tetrode, while the latter estimates the contamination of the spike

cluster with noise/other spikes. Figure III-6 shows means±SEM for isolation distance and L-ratio from recordings undertaken in the familiar environment before any probe trial was conducted. Smaller values for L-Ratio indicate lower contamination of single unit clusters with noise/other spikes and higher values for isolation distance indicate better separation of clusters inside the cluster space.

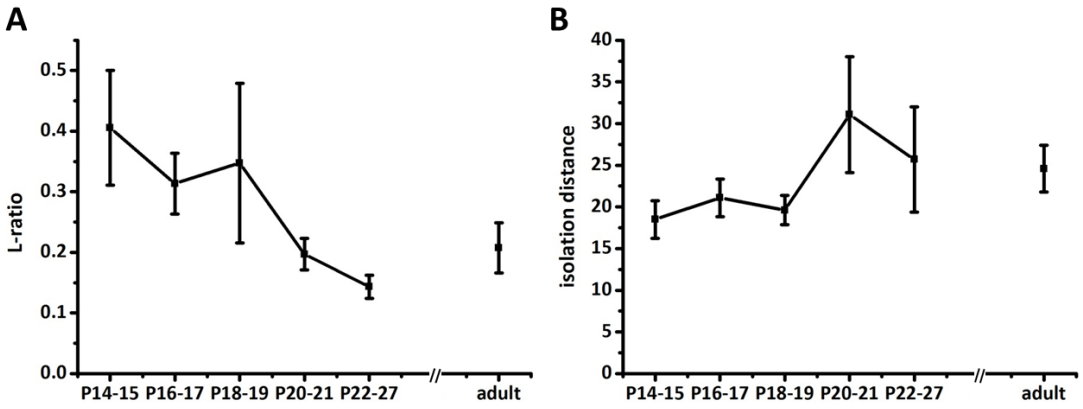


Figure III-6: Quality of signal unit clusters across all age bins. Shown are L-Ratio (A) and isolation distance (B). All values are means±SEM.

Although at least for the L-ratio there seems to be a trend to decrease across age (P14-14: 0.41±0.1, adults: 0.21±0.04), neither measure shows a main effect of age in a statistical analysis (ANOVA; isolation distance: $F_{5,485}$=1.39, p=0.23; L-ratio: $F_{5,506}$=1.04, p=0.39). To exclude conclusively that this trend in decreasing L-ratios across age bins might nonetheless bias the results, L-ratios were correlated to spatial information for the bins with the highest mean L-ratios (P14-15, P16-16 and P18-19). Figure III-7 clearly demonstrates that there is no relation between the average L-ratio of a single unit cluster and its average spatial information content for these age bins. It can thus be concluded that this will not confound the results of the subsequent data analyses.

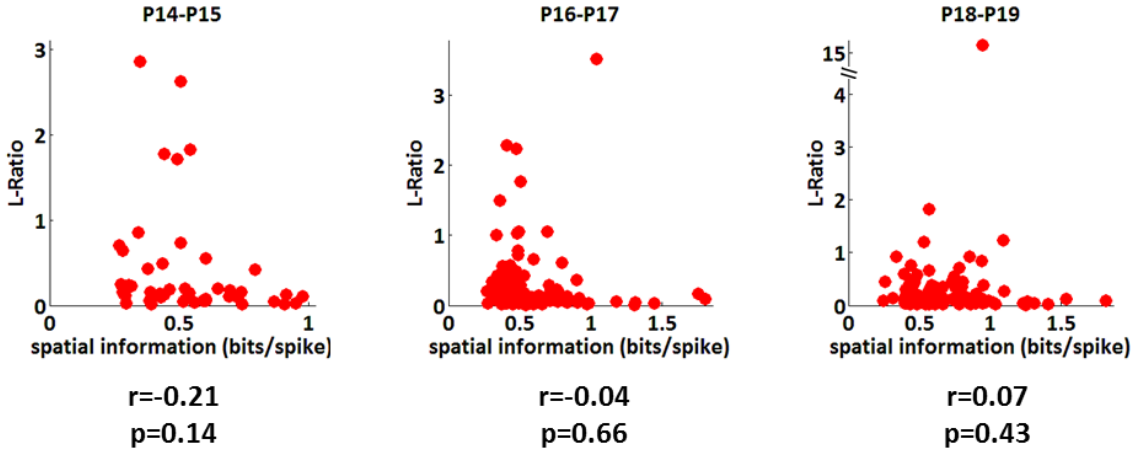


Figure III-7: Scatterplots showing that there is no correlation between L-ratio and spatial information for P14-15, P16-17 and P18-19. Each red dot represents one place cell. Depicted are average L-ratios and spatial information scores across the recording trials in the familiar environment before any probe trial was run. Correlation coefficients (r) and p-values (p) are indicated below scatterplots. Note y-axis break in plot for P18-19.

III.2.3 Anatomical locations of recordings in CA subfields (CA1 vs. CA3)

Before describing the basic properties of place cells recorded in the familiar environment across development, one important point concerns the brain region from which these cells were recorded. Most place cells in this study were recorded from the CA1 region of the hippocampus proper (P14-P21: n=276, 75% of all place cells recorded across these age bins). However, a smaller subset between P14-P21 was recorded from CA3 (P14-P21: n=67, 18% of all place cells recorded across these age bins). No place cells were recorded from CA3 in animals older than P21. In one animal the recording site was situated at the border between CA1 and CA3 (see Figure III-44). This dataset was excluded from the comparison of place cell properties across CA subfields (this dataset accounts for the remaining 7% of the place cell

population). Before pooling data obtained from CA1 and CA3 for the subsequent analyses, it is important to demonstrate that place cells recorded from both CA subfields do not differ across the parameters measured throughout this thesis. These are quality (spatial information, spatial coherence and intra-trial correlation) and stability (inter-trial correlation and centre of mass shift between trials) of the spatial signal, as well as some basic properties like firing rates (mean and peak firing rates), average size of the main firing field and number of place fields per cell. The following figures (see Figure III-8, Figure III-9, Figure III-10 and Figure III-11) show a comparison of the means for the above mentioned parameters for units recorded from CA1 and CA3, while animals explored the familiar environment before any probe was conducted.

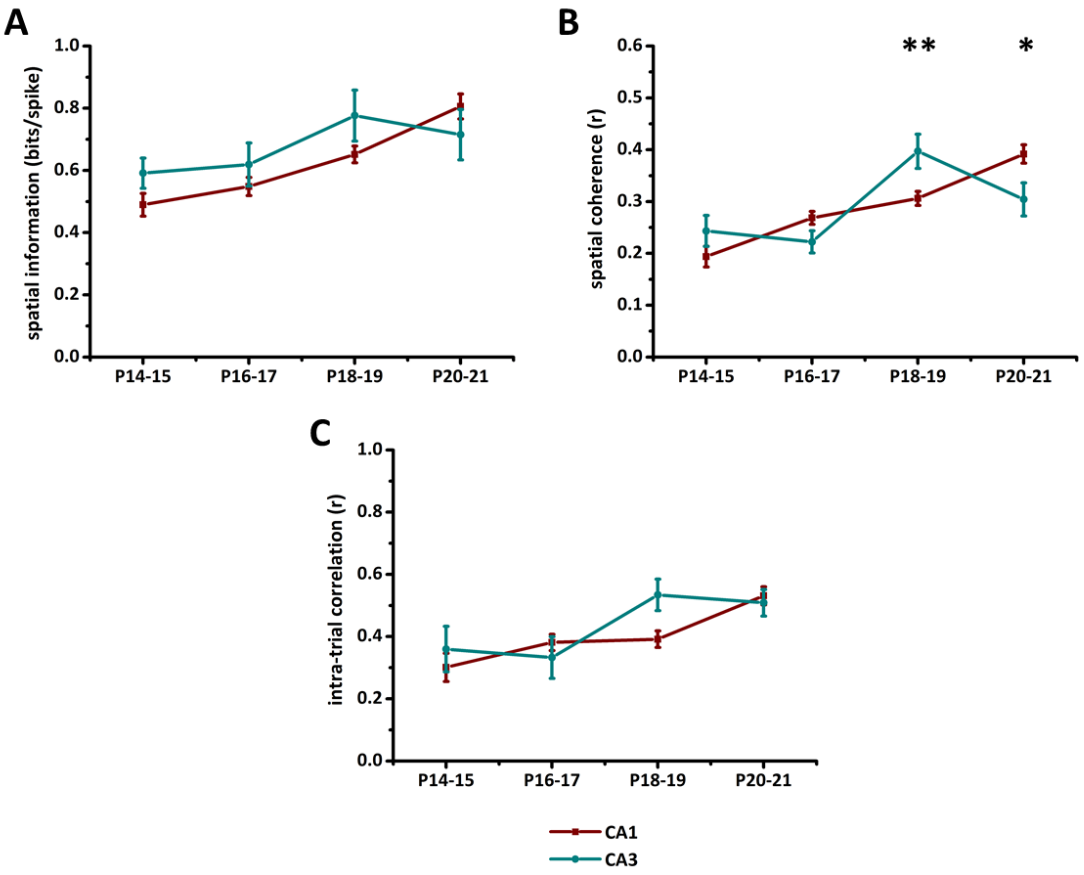


Figure III-8: Quality of the spatial signal of place cells recorded from CA1 (wine) and CA3 (dark cyan) between P14-15 and P20-21. Depicted are spatial information (A, bits/spike), spatial coherence (B, r) and intra-trial correlation (C, r). Values are means from recordings inside the familiar environment before any probe trial was run. All values are population means±SEM. *p<0.05, **p<0.01

A MANOVA for within-trial measures (spatial information, spatial coherence, intra-trial correlation, mean and peak firing rate, field size, number of firing fields) with a 2-by-4 design (brain region by age bin) reveals a main effect of age ($F_{21,993}$=3.09, p<0.001), no effect of brain region ($F_{7,329}$=0.9, p=0.5) and a significant interaction between age and brain region ($F_{21,993}$=2.22, p=0.001). The main effect of age and the underlying between-subjects effects will be discussed in more detail for the analysis of place cell properties in recording trials inside the familiar environment in the following section (see section III.3).

The between-subjects effects for the interaction between age and brain region are non-significant for spatial information ($F_{3,335}$=1.37, p=0.25) and intra-trial correlation ($F_{3,335}$=1.26, p=0.29), while the interaction between the two factors is significant for spatial coherence ($F_{3,335}$=5.61, p=0.001). The pairwise comparisons for the latter measure show significant differences between spatial coherence values at P18-19 (p=0.004) and P20-21 (p=0.02), respectively.

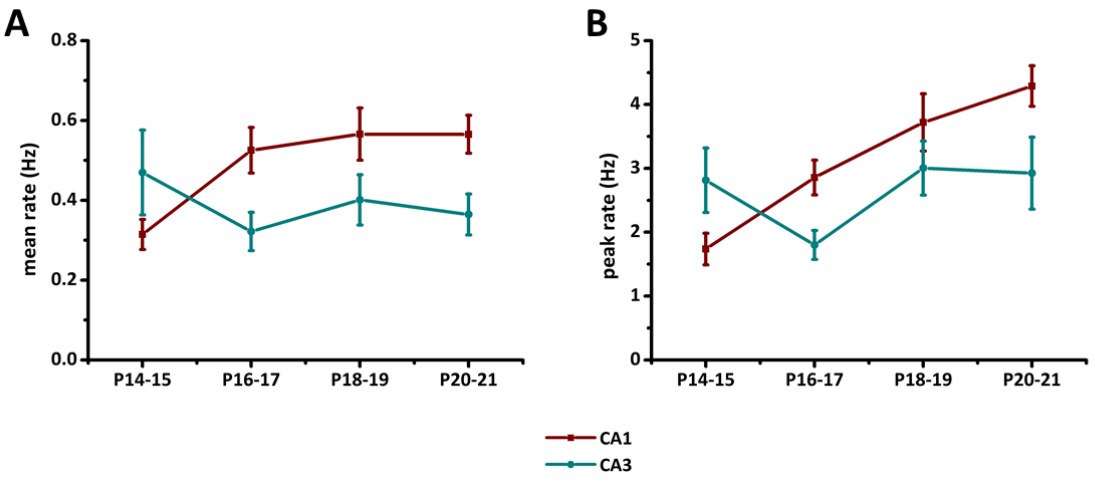


Figure III-9: Firing rates of place cells recorded from CA1 (wine) and CA3 (dark cyan) between P14-15 and P20-21. Depicted are mean firing rate (A, Hz) and peak firing rate (B, Hz). Values are means from recordings inside the familiar environment before any probe trial was run. All values are population means±SEM.

Although there seems to be a trend for generally higher firing rates in CA1 (see Figure III-9), these differences between CA1 and CA3 place cells do not reach statistical significance. The between-subjects effects for the interaction between age and brain region are both non-significant for peak ($F_{3,335}$=1.39, p=0.25) as well as for mean firing rate ($F_{3,335}$=1.39, p=0.25).

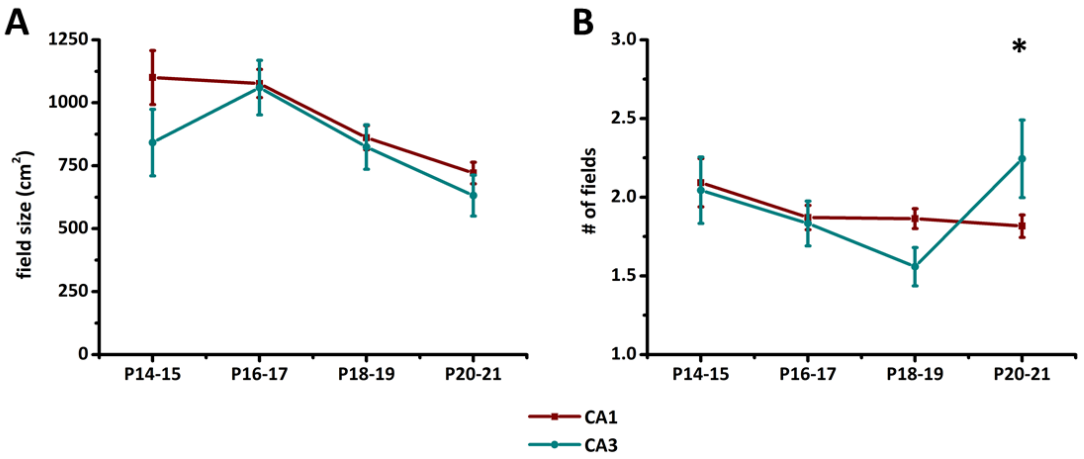


Figure III-10: Field properties of place cells recorded from CA1 (wine) and CA3 (dark cyan) between P14-15 and P20-21. Depicted are mean place field size (A, cm^2) and number of firing fields per cell (B). Field size corresponds to the area of the main firing field. Values are means from recordings inside the familiar environment before any probe trial was run. All values are population means±SEM. *p<0.05

The properties of place fields in CA1 and CA3 in terms of size of main firing field and average number of subfields per cell show a similar change across development in both regions (see Figure III-10). The size of the main firing field is almost identical for both CA subfields at all age bins (interaction age*brain region: $F_{3,335}$=0.65, p=0.57). In both CA subfields field sizes decrease steadily across development (see Figure III-10A). The average number of subfields per cell decrease as well across development (see Figure III-10B). There is significant interaction for this parameter between age and brain region ($F_{3,335}$=2.68, p=0.047) and the pairwise comparisons show that average numbers of firing fields differ at P20-21 between CA1 and CA3 (p=0.03). Note that this data point also exhibits a quite large standard error of the mean for CA3 (ca. 0.25), indicating a large variance for the data obtained at this age bin.

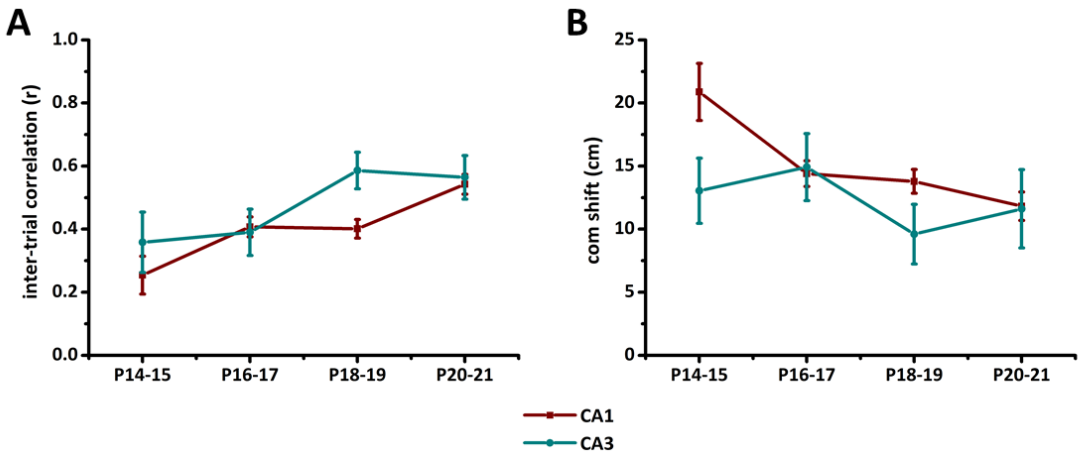


Figure III-11: Stability of the spatial signal of place cells recorded from CA1 (wine) and CA3 (dark cyan) between P14-15 and P20-21. Values are means across recording trials inside the familiar environment before any probe trial was run Depicted are inter-trial correlation (A, r) and centre of mass shift (B, cm). All values are population means±SEM.

A MANOVA for the across-trial measures (inter-trial correlation and centre of mass shift) only reveals a main effect of age ($F_{8,630}$=2.25, p=0.022), while both region and the interaction between age and region are non-significant, respectively (region: $F_{2,314}$=2.23, p=0.11; age*brain region: $F_{6,630}$=1.44, p=0.2). The stability of the spatial signal increases linearly across development in CA1 and CA3 (see Figure III-11A). The average shift of the centre of mass of place fields from trial to trial decreases almost continuously across development in both CA subfields (see Figure III-11B).

From this analysis it is clear that place cell properties do not show any systematic difference across development between CA1 and CA3. That is why these data were pooled for the subsequent analysis of place cell properties obtained from recordings inside the familiar environment.

III.3 Properties of place cells in a familiar environment

III.3.1 Introduction

Before describing the behaviour and properties of place cells during sensory manipulation trials, which will be introduced in a later section (see section III.4), the following section contains a characterisation of the basic properties of place cells across development when recorded in a familiar environment. To assess these properties only data recorded in trials inside the familiar environment before any probe trial was run was taken into account and mean properties were calculated across all trials of interest (see Figure III-12). This section of the thesis will, to some extent, replicate work that has already been published (Langston et al., 2010; Scott et al., 2011; Wills et al., 2010). These analyses are nevertheless important, as i) they provide a check that the basic characterisation of data collected for this thesis matches with data already published and ii) the characterisation described here will extend beyond that which is already published.

Figure III-12 gives a schematic overview of the method of the process of place cell selection and analysis for the characterisation of their properties. As mentioned previously (see sections II.6.4.2 and II.6.4.3, p. 125) the spatial information content of a complex spike cells had to exceed the age-specific threshold (see Figure III-4 and Figure III-12B) in at least one recording trial of a given trial series. For the analysis of place cell properties these trial series (2-3 trials) consisted of recording trials inside the familiar environment before any probe trial was run (see Figure III-12A). For each cell a mean for each property was calculated across those trials (see Figure III-12C). For scores that consist of across-trial measures (inter-trial correlation and

centre of mass shift) these means were calculated by averaging scores for individual cells across the comparisons trial 1 vs. trial 2 and trial 2 vs. trial 3.

The cell numbers for each age bin included in the analysis of place cell properties were already presented in Table III-4.

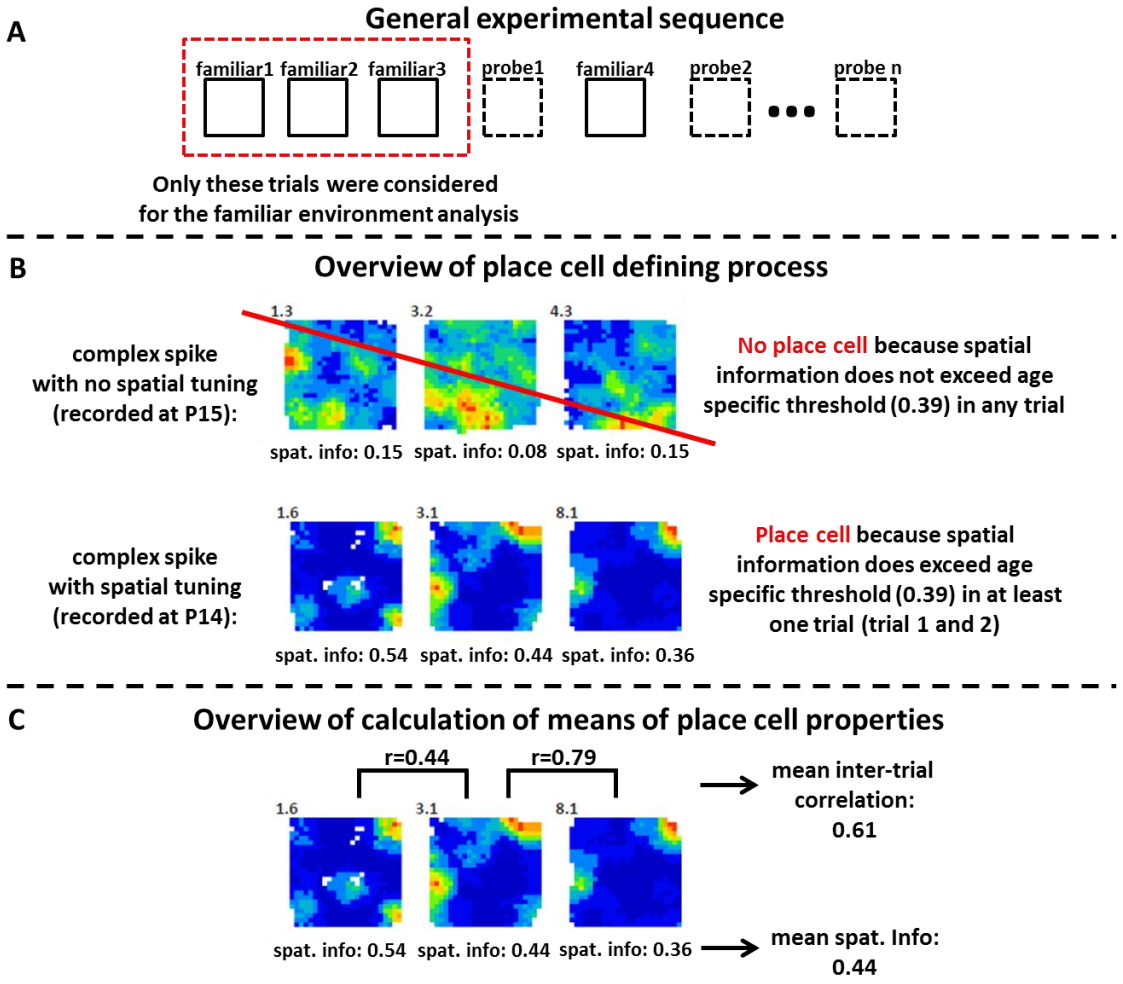


Figure III-12: Overview of process of place cell selection and data analysis. A: general experimental design. Red box indicates trials that were considered for the analysis of place cell properties obtained from recordings inside the familiar environment. B: overview of place cell defining process. Shown are two rate maps of complex spikes cells across three trials in the familiar environment (corresponding to red box in A). Top example is not a place cell, while bottom example is a place cell. C: overview of calculation of cell means for across-trial (top; inter-trial correlation as example) and within-trial measures (bottom; spatial information as example). spat. info: spatial information

III.3.2 Qualitative description of place cell properties in a familiar environment

Figure III-13 shows some representative example rate maps (cells with an average spatial information score across the familiar trials (before any probe was conducted) in the range of the corresponding age mean±standard deviation) for place cells recorded inside the familiar environment for all respective age groups. Depicted are consecutive recording trials inside the familiar environment ('familiar 1-3') for three place cells for each age bin. Note that for cells 2 and 3 in the adult dataset only two trials in the familiar environment are available. The rate maps indicate that there is a developmental change in the quality of the spatial firing. At young ages place cells often exhibit one to several additional firing fields to the main field which can change dynamically across trials (P14-15: cells 1 and 3; P16-17: cell 3; P18-19: cell 2; P20-21: cell 2; P22-27: cell 2). This rarely occurs in adult animals. Furthermore, these rate maps show how the firing fields of place cells become more and more adult-like throughout development. While at young ages place fields are often a bit patchy and contain 'blobs' with higher and lower rates (e.g. P14-15: cell 3; P16-17: cell 2 and 3; P18-19: cell 1; P20-21: cell 1), fields become much more homogenous throughout development with a clear centre of high intensity and a surrounding with progressively lower firing rates. For post-weaning pups the fields already look very similar to adult ones, but can still contain patches of above background outfield firing (e.g. cell 2 in 'familiar3').

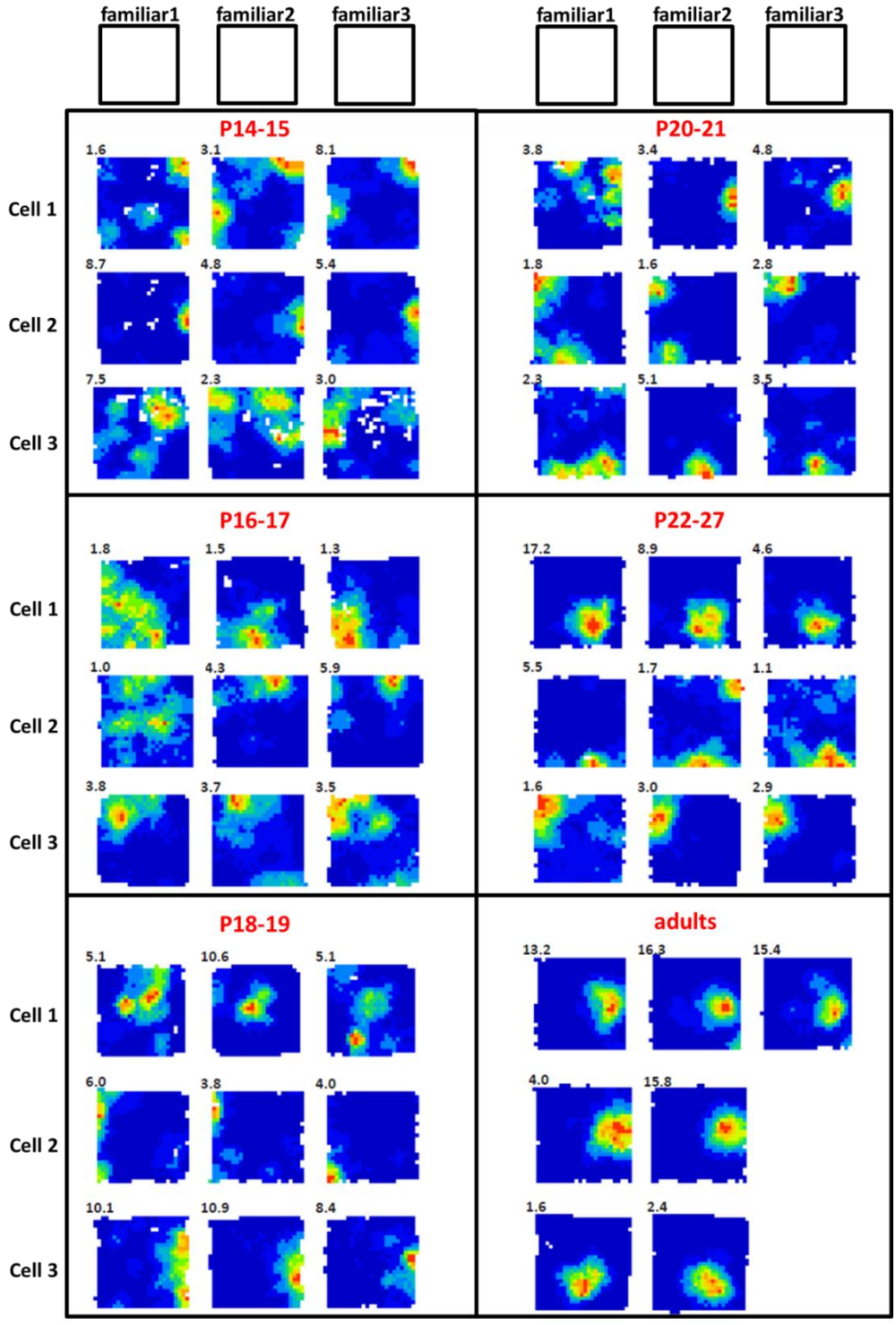


Figure III-13: Representative rate maps of place cells recorded inside the familiar environment across development. For each age bin (black squares) 3 representative examples (average spatial information in range of age mean±standard deviation) are shown across several consecutive recording trials in the familiar environment ('familiar 1-3'). Top row indicates trial sequence. Rate maps are false colour auto-scaled heat maps with progressively warmer colours indicating progressively higher firing rates. Peak firing rates (Hz) are indicated at top left corner of each rate map.

III.3.3 Population analysis of place cell properties in a familiar environment

These qualitatively described developmental changes in terms of the quality and stability of the spatial signal of place cells can be quantified by comparing population means across all three familiar trials for the whole place cell population (see Figure III-14 and Figure III-17).

The statistical analysis (MANOVA) for within-trial scores (spatial information, spatial coherence, intra-trial correlation, mean and peak rate, main field size, number of firing fields) reveals a main effect of age ($F_{35,2555}$=10.62, $p<0.001$). Similar results are obtained by a MANOVA for the across-trial measures (inter-trial correlation and centre of mass shift) which also reveals a main effect of age ($F_{10,970}$=27.47, $p<0.001$). The individual between-subjects effects for all parameters will be discussed in the following subsections and Table III-5 gives an overview of the results of the post-hoc tests (Tukey's HSD) for all place cell properties. Note that for all measures that correspond to correlational data (spatial coherence, intra-trial correlation, inter-trial correlation) values were z-transformed for the statistical analysis.

III.3.3.1 Quality of the spatial tuning of place cells in a familiar environment across development

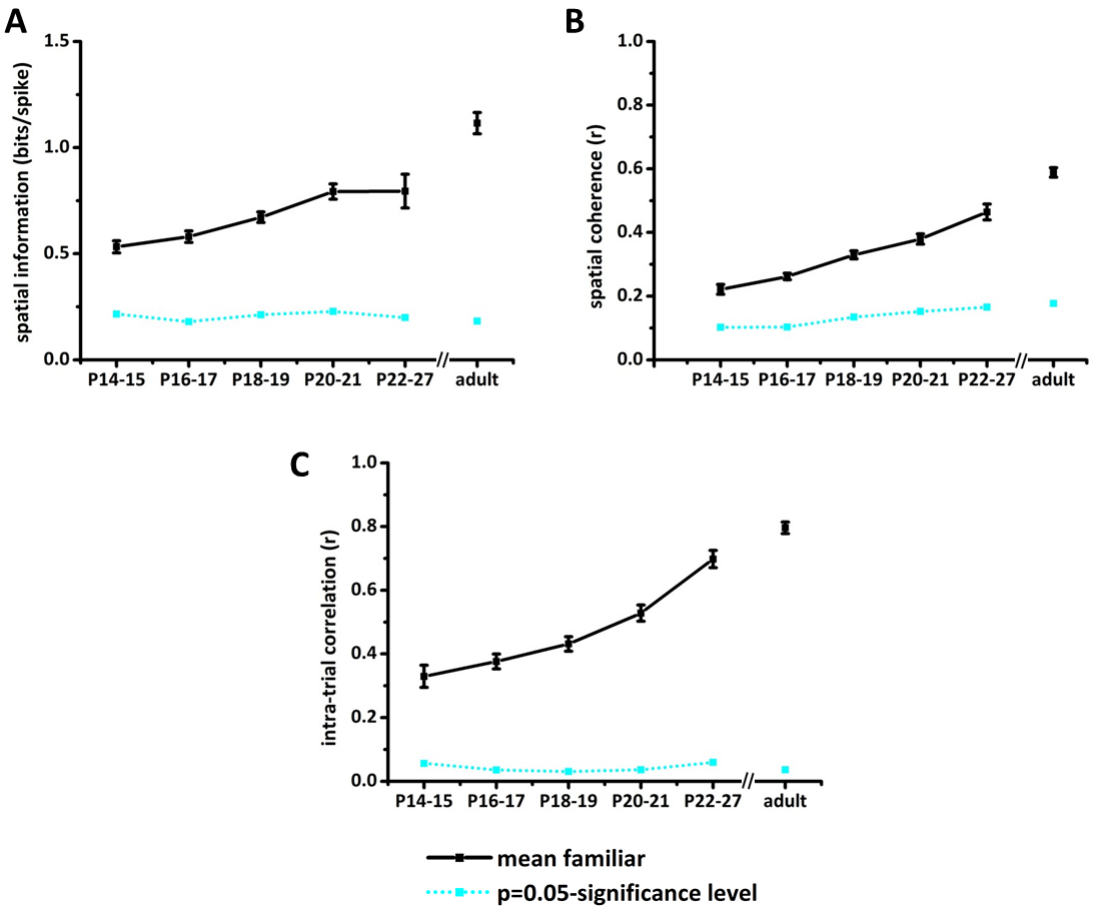


Figure III-14: Quality of the spatial signal of place cells (black lines) across development (P14-15 – adults). Depicted are spatial information (A, bits/spike), spatial coherence (B, r) and intra-trial correlation (C, r). The dotted cyan line depicts the 0.05-significance level of expected mean values of measures based on spike-shuffled data. All values are means±SEM and refer to recording trials inside the familiar environment before any probe was run.

Average spatial information and coherence per place cell both show a steady increase across development (spatial information: $F_{5,513}$=31.32, p<0.001; spatial coherence: $F_{5,513}$=86.64, p<0.001), although adult levels are not yet reached by the end of the 4th week of a rat pup's life (see Figure III-14 and Table III-5). The average intra-trial correlation of place cell firing (see

Figure III-14C) also increases in a linear fashion with age ($F_{5,513}$=79.04, p<0.001). This indicates that within a single trial the location specific firing of place cells becomes more and more reliable, since intra-trial correlation correlates the first to the second half of a recording trial.

At all ages place cells on average show a significantly greater than chance spatial coherence and intra-trial correlation since the 'familiar means' (black lines) exceed by far the 95th percentiles of respective distributions based on spike-shuffled data of expected means of populations of the same size as the original data (cyan dotted line). Since place cells were defined by their spatial information scores it is obvious they should on average exceed this significance level.

III.3.3.2 Firing rates of place cells in a familiar environment across development

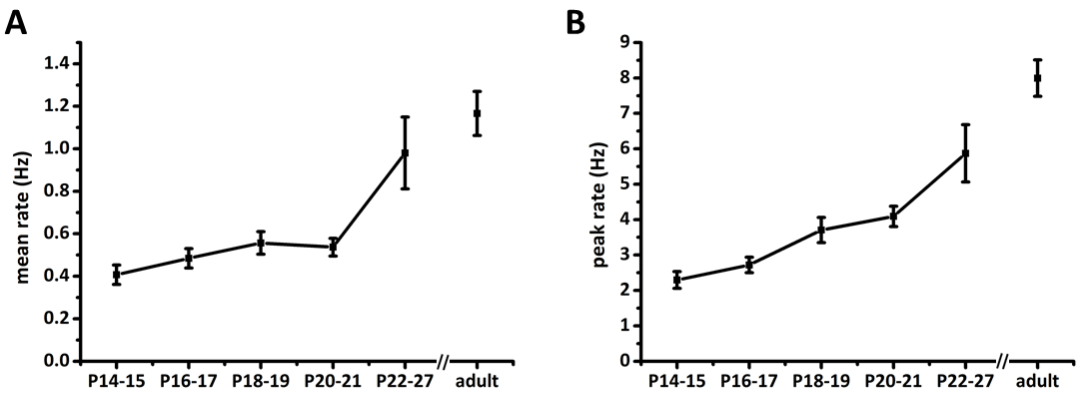


Figure III-15: Firing rates of place cells across development (P14-15 – adults). Depicted are mean firing rate (A, Hz) and peak firing rate (B, Hz). All values are means±SEM and refer to recording trials inside the familiar environment before any probe was run.

As already shown for the average firing rates of the complex spike cell population (see Figure III-3 and Table III-2), firing rates of place cells (see Figure III-15) increase continuously across development (mean rate: $F_{5,513}$=16.51, p<0.001; peak rate: $F_{5,513}$=29.68, p<0.001). There are however some differences to the complex spike cell population. For complex spike cells only data recorded at P22-27 shows significantly higher mean firing rates than all other age bins. For place cells on the other hand, mean firing rates also differ between adult controls and all age bins containing animals younger than P22 (see Table III-5). Furthermore there also seem to be some differences between the changes in mean and peak firing rates across development (see Figure III-15). While the latter increases linearly with age the former seems to not change much until post-weaning age, where adult levels are reached (P22-27: 0.98±0.17 Hz, adults: 1.17±0.1 Hz). However, if the age bin including post-weanling rat pups (P22-27) is split up into two day age bins (i.e. P22-23, P24-25 and P26-27) the increase seems to be rather linear as well for the average firing rates of place cells (mean firing rates: P22-23: 0.75±0.24 Hz, P24-25: 0.88±0.19 Hz, P26-27: 1.36±0.45 Hz).

III.3.3.3 Field properties of place cells in a familiar environment across development

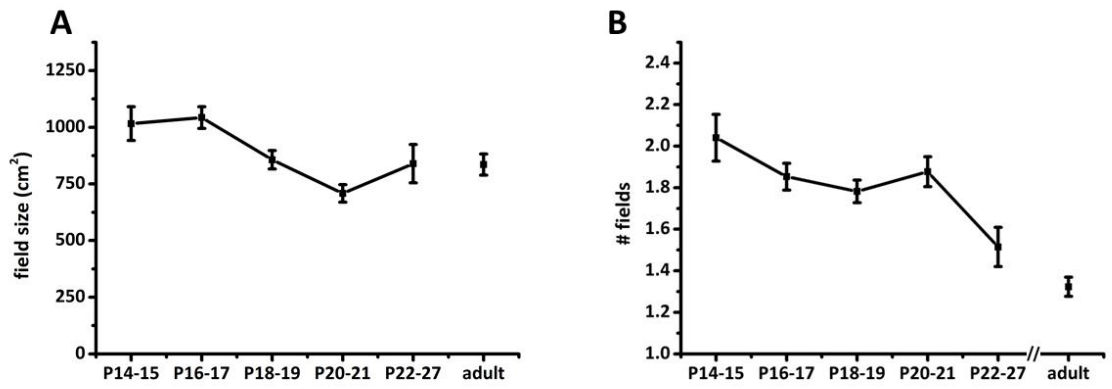


Figure III-16: Field properties of place cells across development (P14-15 – adults). Depicted are mean size of main firing field (A, cm^2) and number of firing fields per cell (B). All values are means±SEM and refer to recording trials inside the familiar environment before any probe was run.

General properties of firing fields of place cells (see Figure III-16) show a change across development (main field size: $F_{5,513}$=6.19, p<0.001; number of fields: $F_{5,513}$=14.95, p<0.001). Place cells in young animals (P14-17) have larger fields than in older animals (P14-15: 1016±75 cm^2, P16-17: 1042±48 cm^2, adults: 836±46 cm^2), while cells at P18-19 have similar sizes to those of adult controls (P18-19: 857±41 cm^2, adults: 836±46 cm^2). For young animals (P14-17) this is also reflected in the lower average spatial information scores for these age bins (see Figure III-14). The average number of subfields per place cell decreases from ca. 2 in pre-weanling rat pups (P14-21) to ca. 1.3 in adults, where the vast majority of place cells only exhibits one place field (see Figure III-16B). Interestingly there is a sharp and sudden drop in the average number of subfields for post-weanling rat pups. In contrast to the changes in mean firing rate (see section III.3.3.2) this is also the case if the age bin 'P22-27' is further split

into two day age bins, although the drop seems to happen rather around P24 than P22 (average number of firing fields: P22-23: 1.77±0.19, P24-25: 1.4±0.14, P26-27: 1.42±0.16).

III.3.3.4 Stability of spatial firing of place cells in a familiar environment across development

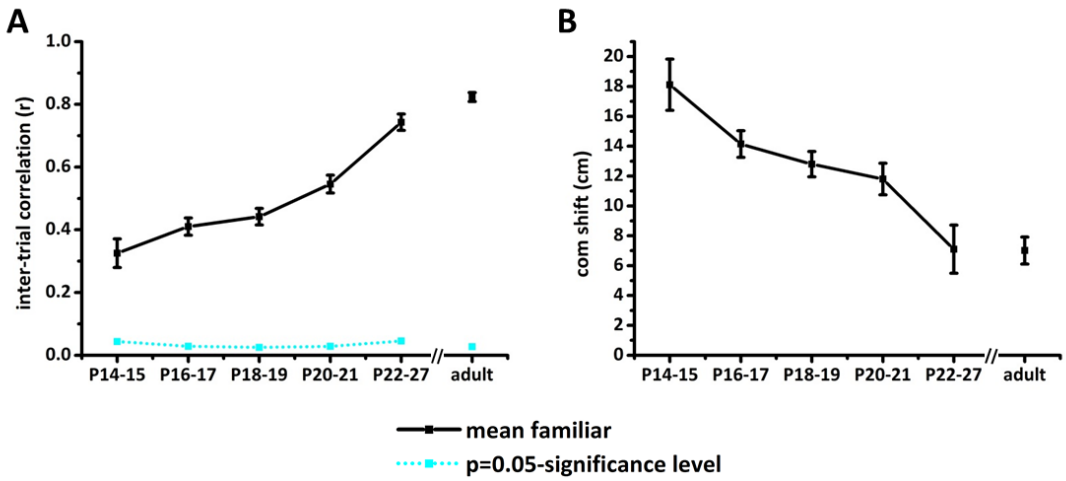


Figure III-17: Stability of the spatial signal of place cells (black lines) across development (P14-15 – adults). Depicted are inter-trial correlation (A, r) and centre of mass shift (B, cm). The dotted cyan line depicts the 0.05-significance level of expected mean values based on spike-shuffled data. All values are means±SEM and refer to recording trials inside the familiar environment before any probe was run.

Not only the quality of the spatial signal (see Figure III-14) is a hallmark feature of place cell firing, also is its stability across multiple sequential recording trials in the same environment (see Figure III-17). This should also be taken into account to assess place cell functioning, particularly because this measure will be completely independent of the place cell selection process (see Figure III-12). The figure shows, just as for the quality of the spatial signal (see Figure III-14), at all ages there are numbers of significantly stable place cells compared to the expected mean values based on spike-shuffled data (compare black and cyan lines in Figure III-

17A). Stability of place cell firing across recording trials improves strongly throughout development ($F_{5,485}$=66.69, p<0.001) and the population means more than double between P14-15 and P22-27 (inter-trial correlation: P14-15: 0.33±0.05, P22-27: 0.74±0.03). While at young ages inter-trial correlation is rather low (until P18-19: < 0.45), stability reaches almost adult-like level in post-weaning rat pups (P22-27: 0.74±0.03, adults: 0.82±0.02), although the difference to adult controls is still significant (see Table III-5). Because inter-trial correlation can be biased by field sizes (large fields inherently tend to have higher inter-trial correlations), another measure of place cell stability was taken into account. It was decided to also look at the average shift of the weighted centres of mass between recording trials (see Figure III-17B). This data shows that at young ages (P14-15) the average shift is rather large with ca. 18±1.7 cm, corresponding to roughly a third of the recording environment width/length (ca. 62 cm). This shift then declines in a linear fashion across development ($F_{5,485}$=12.47, p<0.001) reaching values of around 6-8 cm in post-weanling pups and adults, which for the latter is actually smaller than the typical body length (without tail) of the animals.

III.3.3.5 Summary of statistical analysis of place cell properties in a familiar environment

Table III-5: Overview of post-hoc tests (Tukey's HSD) for the statistical analysis of all place cell properties. All comparisons deal with place cell properties recorded inside the familiar environment. Place cell properties are grouped as in preceding figures. Red values indicate significant p-values (below 0.05-level). Orange values correspond to p-values of exactly 0.05.

spatial information	P14-15	P16-17	P18-19	P20-21	P22-27	adults
P14-15		0.98	0.23	0.001	0.021	<0.001
P16-17			0.42	0.001	0.04	<0.001
P18-19				0.18	0.53	<0.001
P20-21					1	<0.001
P22-27						<0.001

spatial coherence	P14-15	P16-17	P18-19	P20-21	P22-27	adults
P14-15		0.74	0.001	<0.001	<0.001	<0.001
P16-17			0.017	<0.001	<0.001	<0.001
P18-19				0.18	<0.001	<0.001
P20-21					0.042	<0.001
P22-27						<0.001

intra-trial correlation	P14-15	P16-17	P18-19	P20-21	P22-27	adults
P14-15		0.92	0.21	<0.001	<0.001	<0.001
P16-17			0.6	<0.001	<0.001	<0.001
P18-19				0.053	<0.001	<0.001
P20-21					0.002	<0.001
P22-27						0.001

mean rate	P14-15	P16-17	P18-19	P20-21	P22-27	adults
P14-15		0.99	0.82	0.91	0.005	<0.001
P16-17			0.97	1	0.006	<0.001
P18-19				1	0.028	<0.001
P20-21					0.025	<0.001
P22-27						0.76

peak rate	P14-15	P16-17	P18-19	P20-21	P22-27	adults
P14-15		0.99	0.25	0.084	<0.001	<0.001
P16-17			0.37	0.12	<0.001	<0.001
P18-19				0.98	0.041	<0.001
P20-21					0.19	<0.001
P22-27						0.05

main field size	P14-15	P16-17	P18-19	P20-21	P22-27	adults
P14-15		1	0.34	0.003	0.54	0.21
P16-17			0.034	<0.001	0.24	0.013
P18-19				0.2	1	1
P20-21					0.73	0.38
P22-27						1

# fields	P14-15	P16-17	P18-19	P20-21	P22-27	adults
P14-15		0.51	0.15	0.68	0.003	<0.001
P16-17			0.96	1	0.068	<0.001
P18-19				0.89	0.24	<0.001
P20-21					0.048	<0.001
P22-27						0.62

inter-trial correlation	P14-15	P16-17	P18-19	P20-21	P22-27	adults
P14-15		0.57	0.19	<0.001	<0.001	<0.001
P16-17			0.95	0.007	<0.001	<0.001
P18-19				0.074	<0.001	<0.001
P20-21					0.001	<0.001
P22-27						0.015

com shift	P14-15	P16-17	P18-19	P20-21	P22-27	adults
P14-15		0.19	0.023	0.005	<0.001	<0.001
P16-17			0.91	0.53	0.004	<0.001
P18-19				0.98	0.036	<0.001
P20-21					0.16	0.006
P22-27						1

Table III-5 summarises the results of the post-hoc analysis (Tukey's HSD) for all multiple comparisons for age. The results of the statistical analysis show that data obtained from pups aged P14-15 and P16-17 have similar properties for nearly all measures and are different from nearly all scores for older animals. Data recorded at P18-19 and P20-21 shows comparable properties and is generally significantly different for data from post-weanling pups and adult

controls as well as younger animals. For P22-27 the data shows mixed differences to adult controls as some measures already reached adult levels by that age (mean rate, field sizes, number of firing fields and centre of mass shift) while others still differ significantly (spatial information and coherence, intra- and inter-trial correlation). In general this analysis shows that throughout development nearly all measures seem to change/improve in a very coherent fashion reaching adult-like levels during the fourth postnatal week for some, but not all place cell properties.

III.3.4 Place cells are stabilised by boundaries in pre-weanling rat pups

Figure III-17 showed that on average inter-trial correlations of place cells is relatively low for rat pups aged P19 or younger (< 0.45; ca. half of adult values). As stability of place cell firing across recording trials in the same environment is probably the hallmark feature of place cells in adult rats, some additional analysis was performed in an attempt to understand whether any factor can be identified that predicts higher place cell stability in young rat pups. If the data for place cell stability is analysed with respect to the position of individual firing fields inside the recording environment an interesting relationship becomes apparent. There is a strong correlation between inter-trial correlation and distance of the centres of mass of the main firing field from the nearest environmental boundary for rat pups aged P21 or younger (see Figure III-18). Before weaning place cells with fields close to an environmental boundary tend to be more stable than those with fields further away from the walls. In contrast place cells recorded from post-weanling rat pups or adult control animals do not show this effect. Here, a shorter distance of a place field to the nearest environment wall does not predict a higher average stability of the place field across recording trials. This change seems to happen fairly abruptly around the beginning of the 4th week of a rat pup's life.

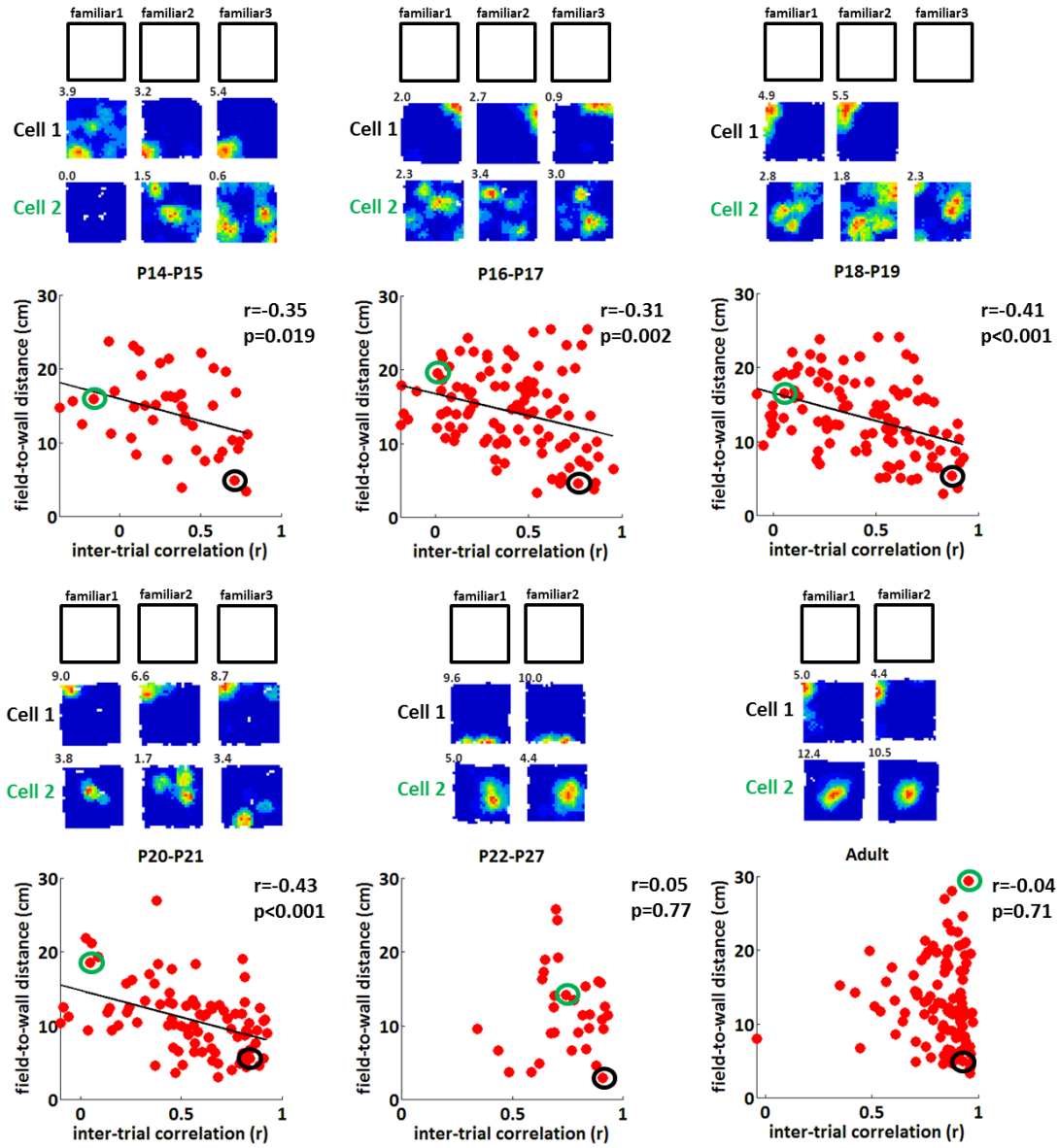


Figure III-18: Correlation of place cell stability with field-to-wall distance in pre- but not post-weanling rat pups or adults. For each age bin, scatterplots with inter-trial correlation over field-to wall distance (measured by distance of centre of mass of place field to nearest environmental boundary) are presented. At top right of each scatterplot correlation coefficients (r) and p-values are indicated. In case of a significant correlation ($p<0.05$) a linear fit of the data (black line) is shown. Above each scatterplot two representative example rate maps (one with a field close to the walls (cell 1) and one with a field far from walls (cell 2)) are shown. Example font colour corresponds to circled data point colour in scatterplot. Numbers at top left corners of rate maps indicate peak firing rate (Hz).

Interestingly, spatial information and size of main firing field are also strongly correlated with the average field-to-wall distance (data not shown, see Table III-6), albeit not only for young rat pups, but for all ages including adults, which reflects that this is an inherent feature of place cells independent of the age of the animal. However, only the trial-to-trial stability shows a relation to closeness-to-walls that is specific to development.

Table III-6: Correlation of field-to-wall distance to spatial information (top rows) and main firing field size (bottom rows). Correlation coefficients (r) and p-values are given. Red p-values indicate significant correlations (<0.05).

spatial information	P14-15	P16-17	P18-19	P20-21	P22-27	adults
r	-0.32	-0.54	-0.40	-0.36	-0.46	-0.43
p	0.026	<0.001	<0.001	<0.001	0.006	<0.001
main field size						
r	0.74	0.80	0.64	0.74	0.82	0.64
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

One potential artefactual source of this effect is that the post-weanling dataset is smaller than the other age bins (see Table III-4). One might thus argue that this is the reason for an absence of a significant correlation between stability and field-to-wall distance for this data point. To control for this, the remaining age bins were randomly subsampled with the same number of cells as for P22-27. This procedure was repeated 10,000 times for each age bin. The means of the resulting distributions of r values (mean $r_{Distribution}$) were then compared to the original r values ($r_{original}$) obtained from Figure III-18 (see Figure III-19). For all distribution the differences between $r_{Distribution}$ and $r_{original}$ yield very small values. The differences are 0.0014 for P14-15, -0.0016 for P16-17, 0.0038 for P18-19, 0.0013 for P20-21 and -0.0236 for adult controls. This shows that even with subsampled data the average correlation coefficients hardly differ from

what was found for the original data. Therefore any effect of the lower cell number for data obtained at P22-27 on the absence of a significant correlation can be rejected.

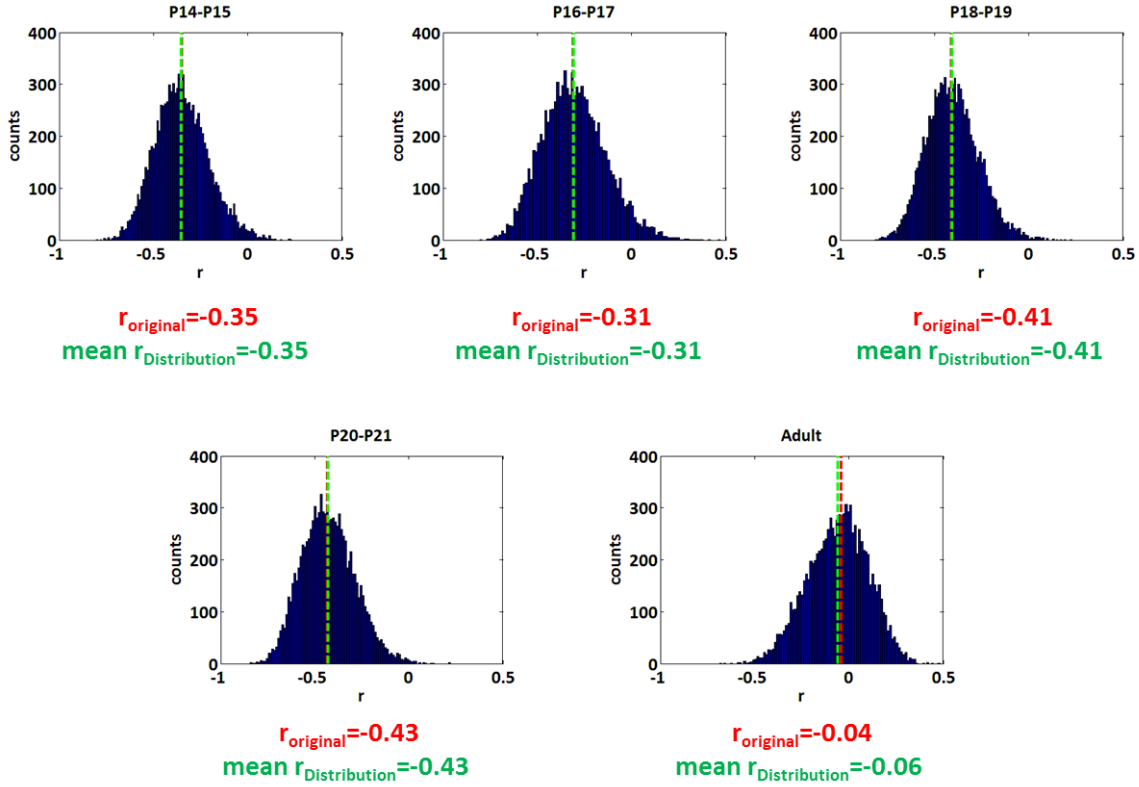


Figure III-19: Distributions of r values from repeatedly subsampling the data in Figure III-18 by the number of cells in P22-27. Depicted are histograms with 10,000 samples each for all age bins (except P22-27). The r values were obtained by randomly subsampling the data from Figure III-18 by the number of cells for data obtained at P22-27. Green dashed line indicates mean r of distribution ($r_{Distribution}$) and red dashed lines indicate r values from Figure III-18 ($r_{original}$). Note that due to the strong overlap between both r values only the green line is visible in some histograms. Both r values are indicated below each histogram. Bin size for histograms is 100.

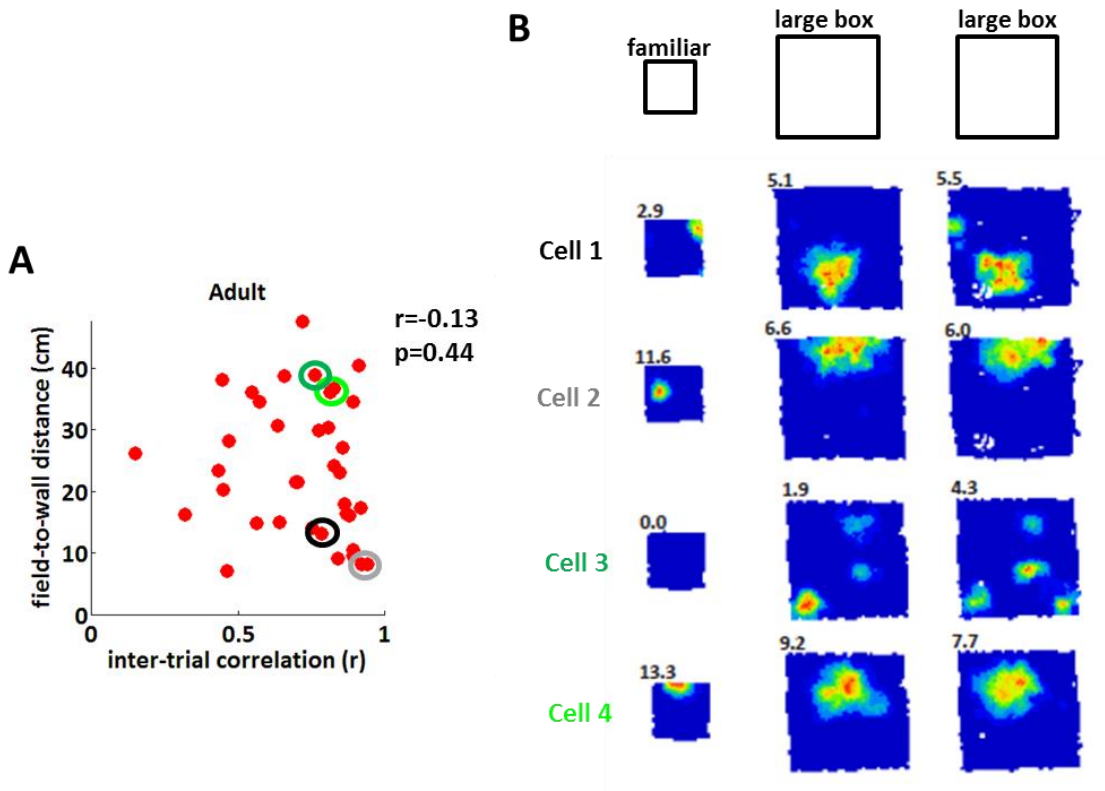


Figure III-20: Correlation of place cell stability with field-to-wall distance in a large environment for adult controls. A: scatterplot with inter-trial correlation over field-to wall distance (measured by distance of centre of mass of place field to nearest environmental boundary). At top right of scatterplot correlation coefficient (r) and p-value is indicated. B: representative example rate maps (two with fields close to walls (cell 1 and 2) and two with fields far from walls (cell 3 and 4)). Rate maps show activity in familiar environment (left column) and two trials in the large box (middle and right column). Example font colour corresponds to circled data point colour in scatterplot. Numbers at top left corner of rate maps indicate peak firing rate (Hz).

Another important consideration relates to the body size of the animals. At least for the adult controls one might argue that proportionally the environment is much smaller than for rat pups. This means that at any point in the environment an adult animal will always be relatively closer to a boundary than a small rat pup. That is why for a subset of the adult animals (n=3) a control experiment was conducted where place cells were recorded in a much larger environment (side length familiar environment: ca. 62 cm, side length large box: ca. 122 cm). As can be seen in the figure (Figure III-20A), even in an environment with an area four times as

large as the standard familiar box, place cell stability in adult controls does not correlate with distance of the place field centres of mass to the nearest boundary. Place cells recorded inside the large box do have a slightly lower average inter-trial correlation compared to the average for adults inside the familiar environment (large box: r=0.71±0.03; familiar environment: r=0.82±0.02), but still larger than for any of the age bins where the correlation between stability and field-to-wall distance is found (compare to Figure III-17A). These results further support the interpretation that the correlation of field-to-wall distance and place cell stability in rat pups is a genuine developmental effect and is not caused by the different body size of the animals or other non-specific effects.

III.3.5 Summary of place cell properties in a familiar environment

Properties of place cells inside a highly familiar environment show a clear developmental functional maturation (see Figure III-14, Figure III-15, Figure III-16, Figure III-17 and Figure III-18). All properties show some change across age and in fact often seem to follow a more or less linear trend (e.g. spatial information and coherence or intra- and inter-trial correlation). This highlights the developmental change from place cells with rather fuzzy fields to the stable and spatially well-defined firing fields in adult rats. In other words, while in young animals (< P18) place cells show larger fields carrying less spatial information and occasionally remap even across repeated trials in the same environment, a strong functional maturation process regarding these properties occurs until the end of the 4th week of a rat's life. By that time in development place cell properties are already very much adult-like in terms of stability measures, field properties and firing rates (see Table III-5). Note that although the statistical analysis (see Table III-5) shows a significant difference for inter-trial correlation between data obtained at P22-27 and adult controls the actual values are quite similar (see Figure III-17). On the other hand is the quality of the spatial signal (see Figure III-14) still different at P22-27 from adults, which indicates that these properties continue to mature beyond this time point until adult-like levels will be reached. These results replicate those seen in previous work (Langston et al., 2010; Scott et al., 2011; Wills et al., 2010).

Special consideration deserves the reduction in average number of firing fields (see Figure III-16 B) from pre- to post-weanling pups and adults. This seems to be the only property which shows a rather abrupt change around P24, a few days after the time point of grid cell emergence in these animals (Langston et al., 2010; Wills et al., 2010).

However, arguably the most interesting result concerns the dependence of place cell stability on field-to-wall distance (see Figure III-18). Only pre-weanling rat pups show this effect, while it is absent in post-weanling pups and adult rats. Several controls, experimental (see Figure III-20) as well as analyses (see Figure III-19 and Table III-6), were performed to ensure that this constitutes a genuine developmental effect and is not due to the unequal sample size of cells per age bin (especially P22-27), or the large difference in body size between adult rats and rat pups of any age. The results of these controls all validated the effect. This stresses the importance of environmental boundaries for the stabilisation of the place cell representation in young animals and raises the question what might cause the sudden independence of place cell stability from field-to-wall distance in post-weanling animals.

III.4 Sensory integration in place cells throughout development

III.4.1 Introduction

The previous section described the development of place cell properties from pre- to post-weanling rat pups and adult animals, when recorded in a fixed familiar environment. In the introduction it was already mentioned that place cells in adult rats are anchored to one or more sensory cues inside or outside an environment (see section I.3.1.6). These cues can be of various sensory modalities and individual cues can influence the location specific firing of different place cells to variable degrees, although visual cues usually exert the strongest influence. Because the sensory systems of rat pups are only poorly developed at birth and become functional in a sequential way at certain time points thereafter, it is of great interest to assess whether the influence of sensory cues on place cell firing in rat pups is rather different to adult animals. This could for example be a much stronger influence of local olfactory cues (e.g. urine traces on environment floor) on the firing of place cells in young animals or a much reduced influence of visual cues until the visual system has matured to a certain degree.

To assess the influence of sensory cues of different modalities on place cell firing throughout development, experiments were conducted in which one or more parts of the sensory environment were manipulated during (a) probe trial(s). The response of place cells during these probe trials was then compared to the basic properties of place cells recorded in the familiar environment. The manipulations of the recording environment consisted of i) replacing the floor, ii) the walls or iii) both floor and walls for visually identical copies to those

of the familiar environment. The parts of the environment were always replaced by visual replicas, thus only changing the olfactory and tactile intra-maze cues, but not the visual ones. Furthermore some probe trials were conducted in absolute darkness inside the familiar environment. Additional probe trials were run in totally novel environments in light as well as in dark conditions.

Although in the actual experiments usually two probe trials interspersed with familiar trials were run in one session, every probe trial type is analysed separately and compared only to familiar trials that were part of any session including this probe trial type (see Figure III-21A). These trials then formed a 'series'. To define place cells within a series two different criteria were applied depending on which properties were to be analysed (see Figure III-21B, C). For all within-trial scores that describe place cell properties on a single trial level (spatial information, spatial coherence, intra-trial correlation, firing rates, place field properties) a similar approach was taken to the one employed for the analysis of place cell properties in a familiar environment (see Figure III-12). A unit had to exceed the age specific threshold of spatial information (see Figure III-4B) in at least one recording trial of the trial series (see Figure III-21B).

For the analysis of across-trial measures (inter-trial correlation, centre of mass shift) that measure the change of the locational specific firing between trials the criterion was slightly different (see Figure III-21C). A given unit had to exceed i) the age specific threshold in spatial information in any one of the recording trials inside the familiar environment before any probe trial was run (i.e. trials 1-3) and ii) its mean correlation across these trials had to exceed the 95^{th} percentile of the distribution of inter-trial correlations based on spike-shuffled data (see Figure III-22). What is the rationale for these different criteria depending on which property of a place cell is to be analysed?

For analysing general 'spatiality' (within-trial measures) of place cells across different environments it does not matter whether the units of interest are stable across trials or in what environment specifically they exhibit a significant spatial tuning. A unit which e.g. only exhibits a significant spatial tuning during the probe trial (i.e. exceeding the age specific threshold for spatial information), but in none of the familiar trials should be considered for the population-wide analysis just as a unit which shows the opposite behaviour. This is to avoid under- or overestimating the quality of the spatial tuning during probe and/or familiar trials.

For analysing stability of the place cell representation a different set of criteria should be applied because here place cell properties are compared between familiar and probe trials. Two parameters are of major importance concerning unit properties across recording trials inside the familiar environment before any probe trial was run. Place cells should be significantly spatially tuned during these recording trials. Hence, the first criterion is that units for this type of analysis have to exceed the age specific threshold of spatial information (see Figure III-4B) in any one of the familiar trials before a probe trial was conducted. Secondly, place cells should also show some form of baseline stability throughout these recording trials. In the absence of stability in the familiar condition (baseline) no inferences regarding the effect of any sensory manipulation can be drawn. Choosing the 95^{th} percentile of the inter-trial correlation distribution based on spike-shuffled data (same process as for the distribution of spatial information based on spike-shuffled data) should yield a fairly robust criterion as this value indicates only a 5% chance of some random firing being correlated accordingly (see Figure III-22). Figure III-22 shows these distributions for all respective age bins. Due to the fact that the threshold value hardly changes throughout development (values lie between 0.27 and 0.29) it is clear that this is a robust criterion, not affected e.g. by general changes in firing rates across the different age bins. Place cells with an average inter-trial correlation below these

thresholds across all familiar trials before any probe trial was conducted were deemed unstable and not included for the analysis of place cell stability.

When examples of rate maps are presented in the subsequent sections there will always be one example per age bin of a place cell in terms of its overall spatiality and two of place cells which are defined by their spatiality and stability across the first 2-3 'familiar' recording trials. Of course the two populations are largely overlapping so units will often fit both types of criteria.

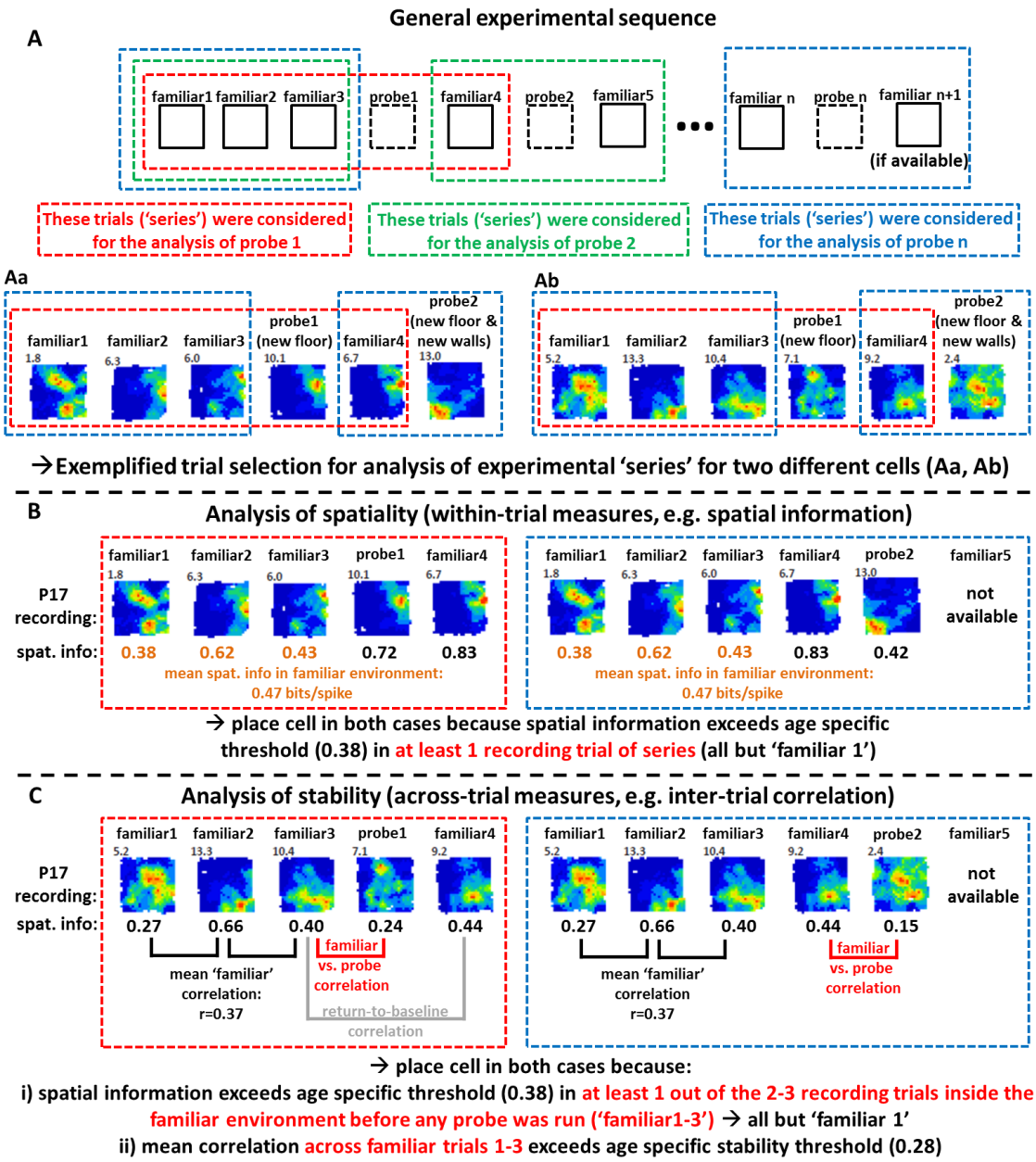


Figure III-21: Schematic overview of data selection process and place cell criteria for the analysis of place cell properties during probe trials. A: general experimental schematic with different coloured boxes indicating trial selection for the analysis of individual probe trials. Aa,b: two example units (both recorded at P17) across a whole experiment. Boxes indicate what trials were selected for the analysis of a probe series. B: overview of place cell defining process for the analysis of within-trial measures for both series selected from Aa. Below rate maps process of mean calculation for the familiar environment is indicated. C: overview of place cell defining process for the analysis of across-trial measures for both series selected from Ab. Below rate maps process of (mean) calculation for the possible inter-trial correlations is indicated. Note that return-to-baseline correlation (grey) will only be calculated if a probe trial is encompassed by two familiar trials. Numbers at top left corner of rate maps indicate peak firing rate (Hz). spat. info: spatial information

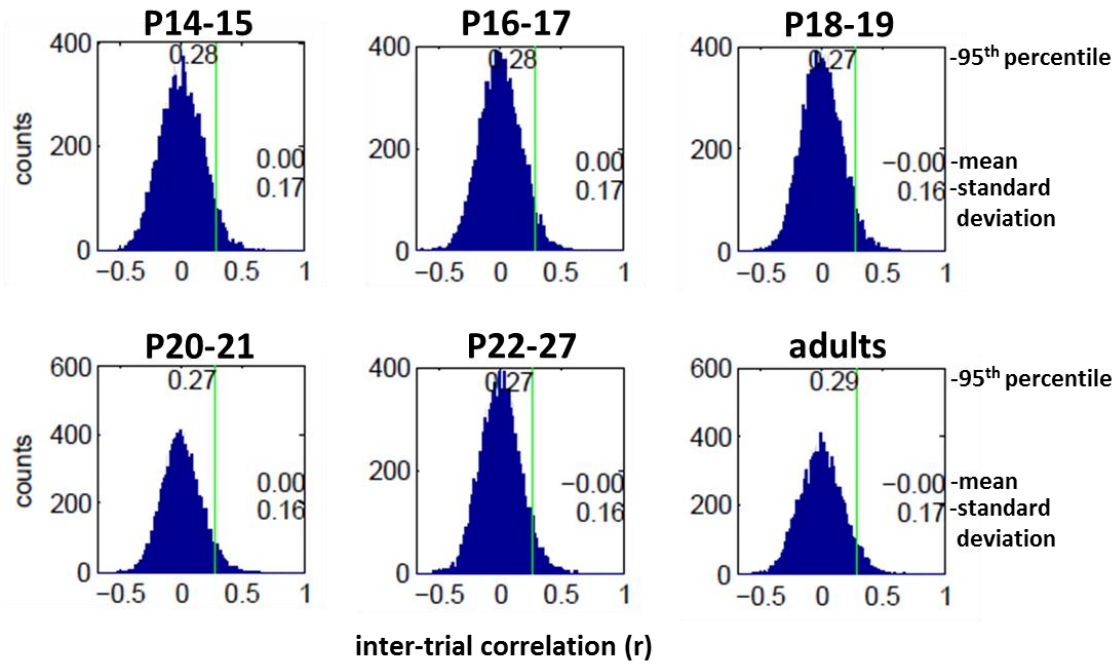


Figure III-22: Overview of distributions of inter-trial correlation based on spike-shuffled data across age bins. Histograms show distributions of inter-trial correlations of shuffled rate maps (20,000 samples per histogram) for all age bins (from top left to bottom right). Green lines depict 95th percentile of distributions (numeric value is indicated at top left to green line) and values in middle right of each histogram show mean (upper value) and standard deviation (lower value) of distributions.

III.4.2 Manipulations of local tactile and olfactory intra-maze cues

III.4.2.1 Effects of replacing the walls of the familiar environment ('new walls')

The first probe that will be discussed in this section of the results is a replacement of the environment walls of the familiar environment. Table III-7 gives an overview of how many complex spike cells were recorded for this probe series, as well as how many of these units were classified as place cells according to their 'spatiality' (see Figure III-21B). Additionally the numbers of place cells after filtering for threshold stability (see Figure III-22, Figure III-21C) are indicated, too. These latter numbers correspond to place cells that are included in the analyses regarding the across-trial measures while the former are included for the analysis of the within-trial measures.

Table III-7: Overview of recorded cell numbers in the 'new walls' probe across development. Given are numbers of complex spike cells (left column), place cells as defined for the analysis of within-trial measures (middle column) and stability filtered place cells for the analysis of the across-trial measures (right column).

	# complex spike cells	# place cells (according to 'spatiality')	# place cells (stability filtered)
P14-15	no cells	no cells	no cells
P16-17	49	15	9
P18-19	32	14	3
P20-21	62	36	24
P22-27	36	18	15
adult	45	44	37

Representative rate maps recorded for the ‘new walls’ probe are shown in Figure III-23. Replacing the walls of the familiar environment has rather little influence on place cell firing, particularly for rats older than P20. Figure III-23 shows three representative place cells at each age bin (note that there is no data for P14-15 for this probe trial type). It is clear from these examples that overall place cells maintain their location specific firing from the familiar trials during the ‘new walls‘ probe. There are some changes to firing fields for the ages P16-19, for example cell 3 at P16-17 shows a strongly enlarged firing field and also cells 1 and 3 at P18-19 show some minor changes of their place fields during the probe trial. Note that the latter two examples maintain these small changes in the subsequent recording trial inside the familiar environment.

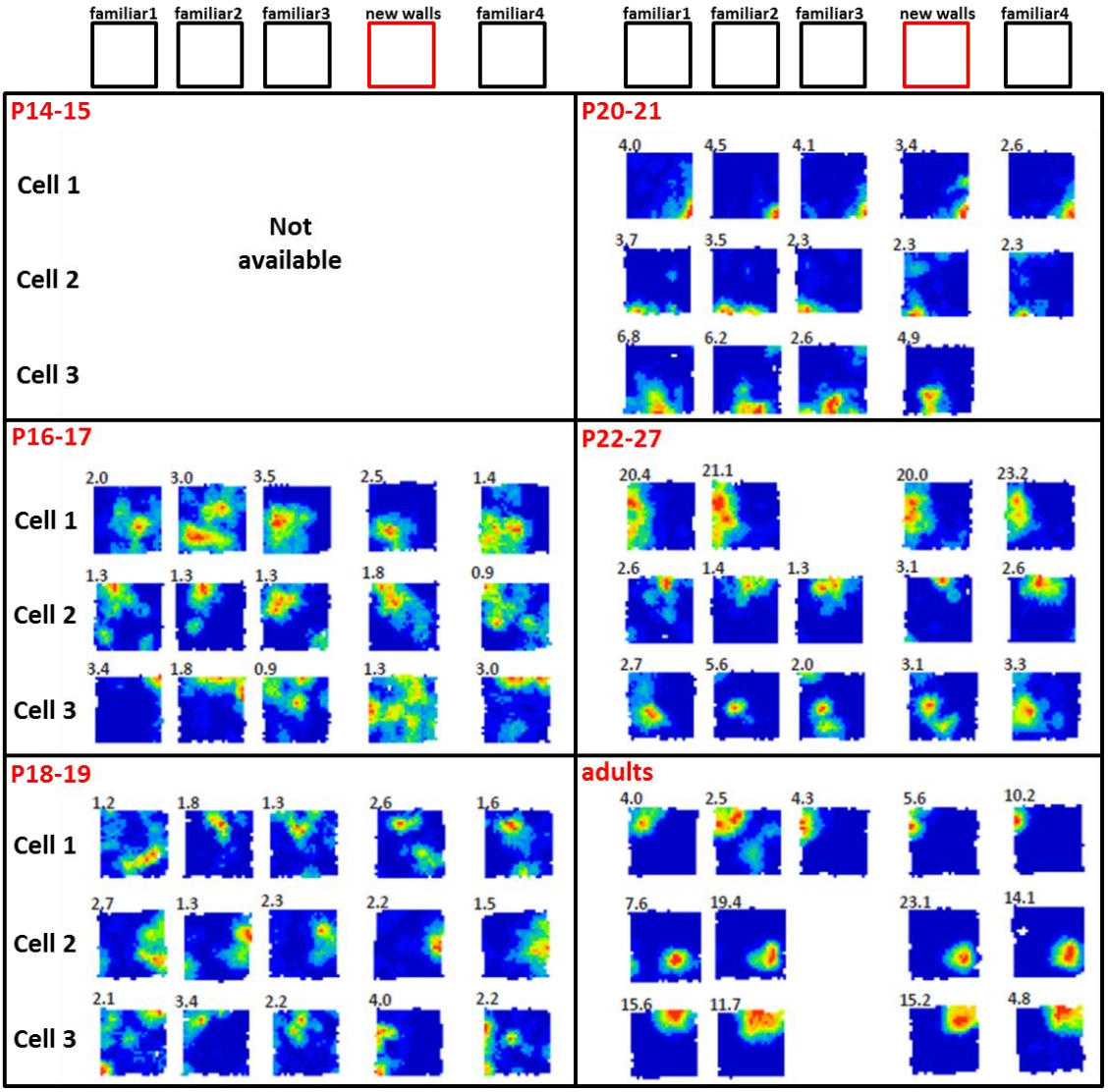


Figure III-23: Representative rate maps of place cells recorded inside the familiar environment and under 'new walls' conditions across development (P16-17 – adults). For each age bin (black squares) 3 representative examples are shown across familiar trials ('familiar1-3') run before the probe trial, the probe trial itself ('new walls') and a following familiar trial ('familiar4'). All example cells shown have average spatial information scores across 'familiar1-3' in the range of age mean±standard deviation. Top row indicates schematic of trial sequence. Rate maps are false colour auto-scaled heat maps with progressively warmer colours indicating progressively higher firing rates. Peak firing rates (Hz) are indicated at top left corner of each rate map. P(x)-(x) indicates age bin. Note that no data is available for P14-15.

Population analysis of place cell properties for the 'new walls' probe

Quality of spatial firing during 'new walls' probe

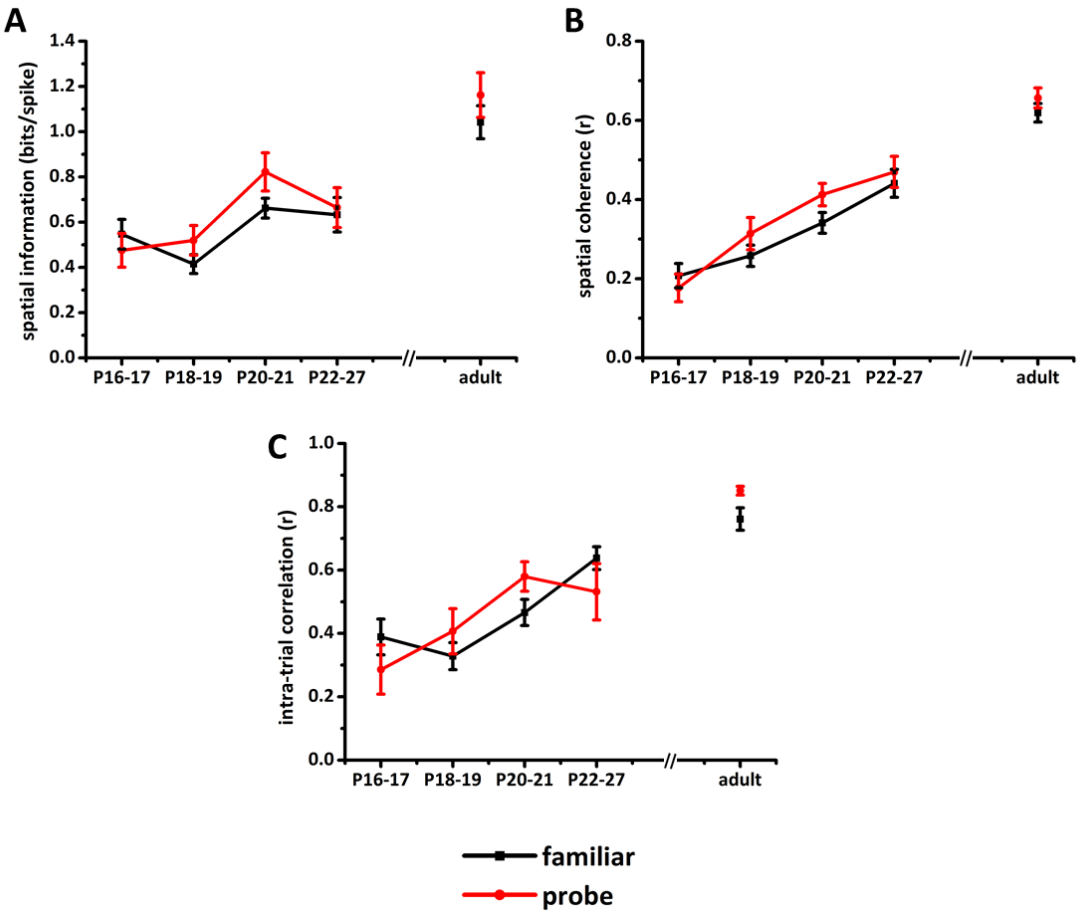


Figure III-24: Quality of the spatial signal of place cells recorded inside the familiar environment (black lines) and under 'new walls' conditions (red lines) between P16-17 and adults. Depicted are spatial information (A, bits/spike), spatial coherence (B, r) and intra-trial correlation (C, r). Values for familiar environment are means across those trials inside the familiar environment before any probe trial was conducted. All values are population means±SEM.

The qualitative description of place cell responses during the probe trial ('new walls') is also reflected in the population-wide analysis of spatiality (see Figure III-24) and stability (see Figure III-25) of place cells. In general the statistical analysis of place cell responses during the

probe trials consists of repeated measures ANOVAs for each individual parameter in an environment by age design.

During the 'new walls' probe place cells show a very similar quality (spatial information and coherence) of the location specific firing as in the baseline familiar trials (see Figure III-24A, B). Both measures increase in a more or less linear fashion across development for both environment types (main effect of age: spatial information: $F_{4,106}=10.36$, $p<0.001$; spatial coherence: $F_{4,106}=36.72$, $p<0.001$) and show comparable values for familiar and probe trials at every age bin (no effect of environment: spatial information: $F_{1,106}=2.08$, $p=0.15$; spatial coherence: $F_{1,106}=3.92$, $p=0.05$). Note however, that the p-value for spatial coherence for the effect of recording environment is exactly at the 0.05-significance level. Furthermore there is no significant interaction between age and environment for both measures (spatial information: $F_{4,106}=1.32$, $p=0.27$; spatial coherence: $F_{4,106}=0.61$, $p=0.65$).

The analysis of intra-trial correlations yields very similar results (see Figure III-24C). There is a steady increase with age ($F_{4,106}=31.46$, $p<0.001$), while the values do not differ between recordings inside the familiar environment and the probe trial ($F_{1,106}=1.82$, $p=0.18$). There is no interaction between the two factors for intra-trial correlation ($F_{4,106}=1.86$, $p=0.12$).

These results clearly show that replacing the walls of a familiar environment has only little impact on the quality of place cell firing at any point in development, with the effect on spatial coherence during the probe trial being a borderline case.

Stability of spatial firing during 'new walls' probe

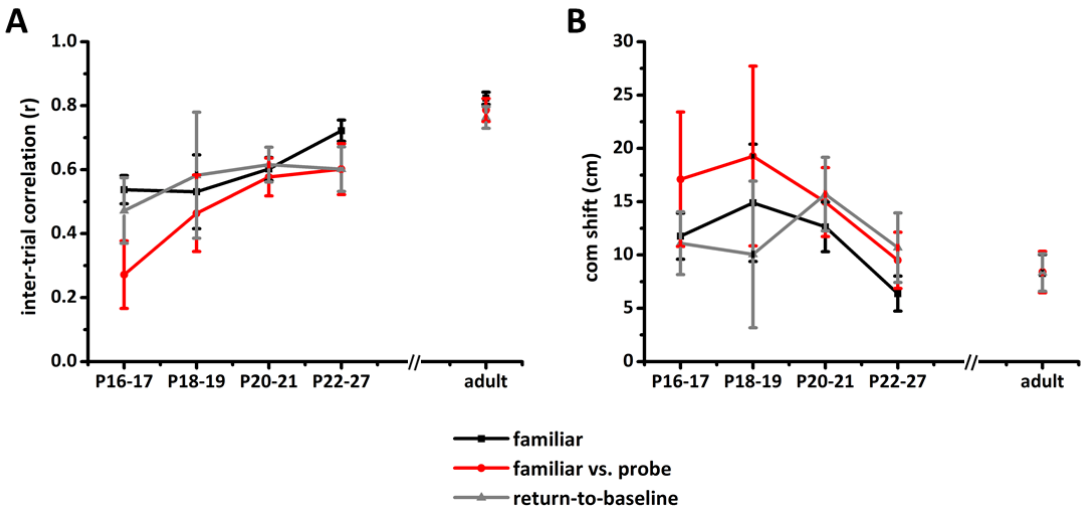


Figure III-25: Stability of the spatial signal of place cells recorded inside the familiar environment (black lines) and under 'new walls' conditions (red lines) between P16-17 and adults. Depicted are inter-trial correlation (A, r) and centre of mass shift (B, cm). The return-to-baseline values (grey lines) indicate the stability of place cells across the two recording trials inside the familiar environment encompassing the probe trial. Values for familiar environment are means across those trials inside the familiar environment before any probe trial was conducted. All values are population means±SEM.

The population analysis of the stability of the spatial firing of place cells during the 'new walls' probe also confirms the qualitative description (compare Figure III-23 and Figure III-25). Inter-trial correlation (see Figure III-25A) generally increases with age ($F_{4,76}$=10.95, p<0.001), indicating higher place cell stability in older animals. Although there appears to be a trend towards lesser stability in the probe at P16-17, there is no significant effect of recording environment ($F_{2,75}$=2.37, p=0.1) and no significant interaction between age and environment ($F_{8,152}$=1.52, p=0.16).

Similar results are obtained for the centre of mass shifts across environments (see Figure III-25 B), apart from an absence of a main effect of age for this parameter ($F_{4,76}$=1.73, p=0.15). Both

the effect of environment as well as its interaction with age are non-significant (environment: $F_{2,75}$=1.42, p=0.25; age*environment: $F_{8,152}$=0.8, p=0.6).

Thus, just as for the quality of the location specific firing replacing the walls of a familiar environment seems to have little influence on place cell stability and induces little or no remapping at most of the age bins. This leads to the conclusion that either animals do not use any local olfactory/tactile cues on the environment walls to anchor place fields or that the remaining local and distal cues are sufficient to re-activate the place cell representation of the familiar environment.

Active vs. inactive cells during 'new walls' probe

One important point concerns cells that become exclusively active during the probe trial or units that specifically cease firing during these trials. The problem with these units is that they are not accounted for when calculating inter-trial correlations or centre of mass shifts for the stability filtered place cell population. Because of that, it is theoretically possible that while the population of active cells maintains stable fields during familiar and probe trial recordings, the population code actually differs to a much larger extent.

Table III-8 gives the absolute numbers and percentages (relative to the whole place cell population) of cells that are either i) active in both familiar and probe ('both environments'), ii) exclusively active during the probe trial ('probe only') or iii) the familiar trials ('familiar only'). Note that these numbers do not necessarily add up to the full count of cells, due to an inconsistent firing pattern of some cells across all familiar trials of a series (e.g. a cell that only fires in one or two familiar trials in a series, but not in other familiar trials). These cells will be ignored for this analysis. The results show that overall the same population of cells is active

during probe and familiar trials at any age bin for the 'new walls' probe. Note that these proportions are calculated for the place cell population as defined by their overall spatiality in a given probe series.

Table III-8: Overview of place cell numbers that are active in both environments and those that are either exclusively active during probe ('probe only') or familiar ('familiar only') trials for the 'new walls' probe. Given are absolute numbers as well as proportion from the place cell population defined by their overall spatiality in a probe series in parentheses. Note that cells showing an inconsistent firing pattern across all familiar trials are excluded from this analysis.

	# both environments (%)	# probe only (%)	# familiar only (%)
P14-15	no cells	no cells	no cells
P16-17	12 (80%)	2 (13%)	0 (0%)
P18-19	14 (100%)	0 (0%)	0 (0%)
P20-21	33 (92%)	0 (0%)	1 (3%)
P22-27	16 (89%)	0 (0%)	0 (0%)
adults	39 (89%)	0 (0%)	1 (2%)

III.4.2.2 Effects of replacing the floor of the familiar environment ('new floor')

After describing the effect or rather absence of any effect on place cell firing after the walls of a familiar environment were replaced, this section presents the effects after the floor was replaced. Table III-9 gives an overview of the respective cell numbers (complex spike cells and place cells) recorded for this type of probe trial across all age bins.

Table III-9: Overview of recorded cell numbers in the 'new floor' probe across development. Given are numbers of complex spike cells (left column), place cells as defined for the analysis of within-trial measures (middle column) and stability filtered place cells for the analysis of the across-trial measures (right column).

	# complex spike cells	# place cells (according to 'spatiality')	# place cells (stability filtered)
P14-15	67	28	13
P16-17	100	46	25
P18-19	85	40	26
P20-21	32	18	14
P22-27	53	28	21
adult	136	112	96

Comparing place cell firing under familiar conditions and when the floor was replaced shows that for this probe trial the location specific firing is influenced to a stronger degree than if the walls were replaced (compare Figure III-23 and Figure III-26). The representative rate maps (see Figure III-26) show that while the majority of the cell population remains unaffected, some cells do remap to some extent during the probe trial (see cell 1 and 2 for P14-15, cell 1 at P18-19, cell 3 at P20-21, cell 2 and 3 at P22-27 and cell 3 for adults). On a qualitative level,

remapping during the ‘new floor’ probe seems to occur at every age bin. The change in place cell firing for those cells affected by the probe ranges from the firing field becoming more fuzzy (cell 2 at P22-27), loss of a well-defined firing field (cell 1 at P14-15 and cell 3 P20-21), ceasing of activity (cell 2 at P14-15 and cell 3 for adults) to moving of the firing field to a completely new location inside the environment (cell 3 at P22-27). Nonetheless, the majority of place cells do maintain their fields during the new floor probe. It is noteworthy to point out that place cells recorded from rat pups seem to show some ‘knock-on’ effects. A ‘knock-on’ effect would be characterised by a change of the place field location/properties in the familiar trial after the probe trial, compared to familiar trials before the probe was run (e.g. a change of firing field location in probe trial that is kept in the following baseline). Cell 1 at P14-15, cell 1 at P16-17, cell 1 and 3 at P18-19, cell 2 at P22-27 all show some differences in their firing fields if the familiar trials encompassing the probe trial are compared. Cell 1 at P14-15 e.g. shows a loss of its well-defined field during the probe trial, which prevails also in the following familiar trial. Cell 1 at P16-17 on the other hand keeps its main field position in both familiar trials encompassing the probe trial, but has an extended field in the trial after the probe. This extended portion seems to have been ‘inherited’ from the probe trial as parts of it are already present during the probe itself.

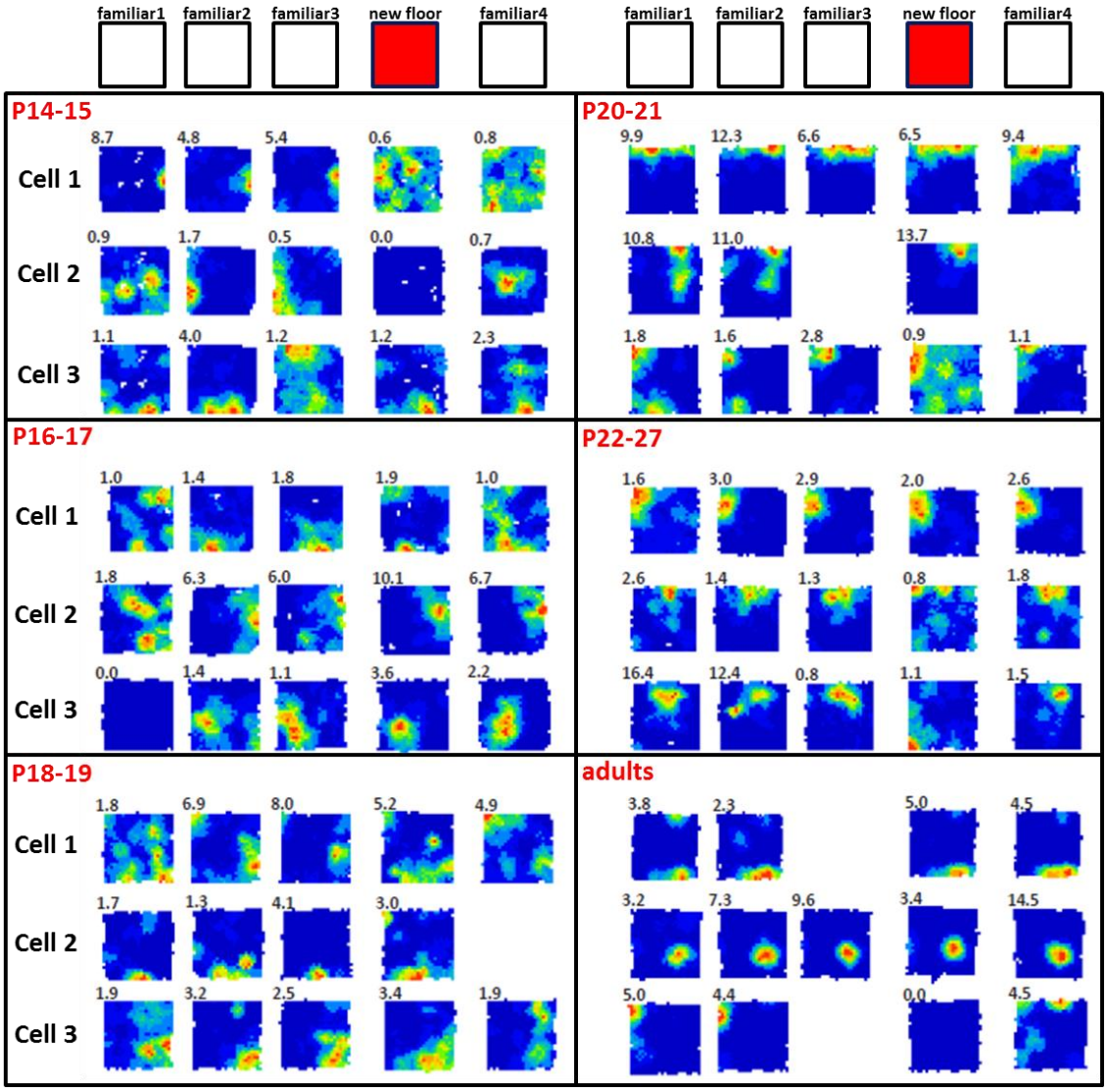


Figure III-26: Representative rate maps of place cells recorded inside the familiar environment and under 'new floor' conditions across development (P14-15 – adults). For each age bin (black squares) 3 representative examples are shown across familiar trials ('familiar1-3') run before the probe trial, the probe trial itself ('new floor') and a following familiar trial ('familiar4'). All example cells shown have average spatial information scores across 'familiar1-3' in the range of age mean±standard deviation. Top row indicates schematic of trial sequence. Rate maps are false colour auto-scaled heat maps with progressively warmer colours indicating progressively higher firing rates. Peak firing rates (Hz) are indicated at top left corner of each rate map. P(x)-(x) indicates age bin.

Population analysis of place cell properties for the 'new floor' probe

Quality of spatial firing during 'new floor' probe

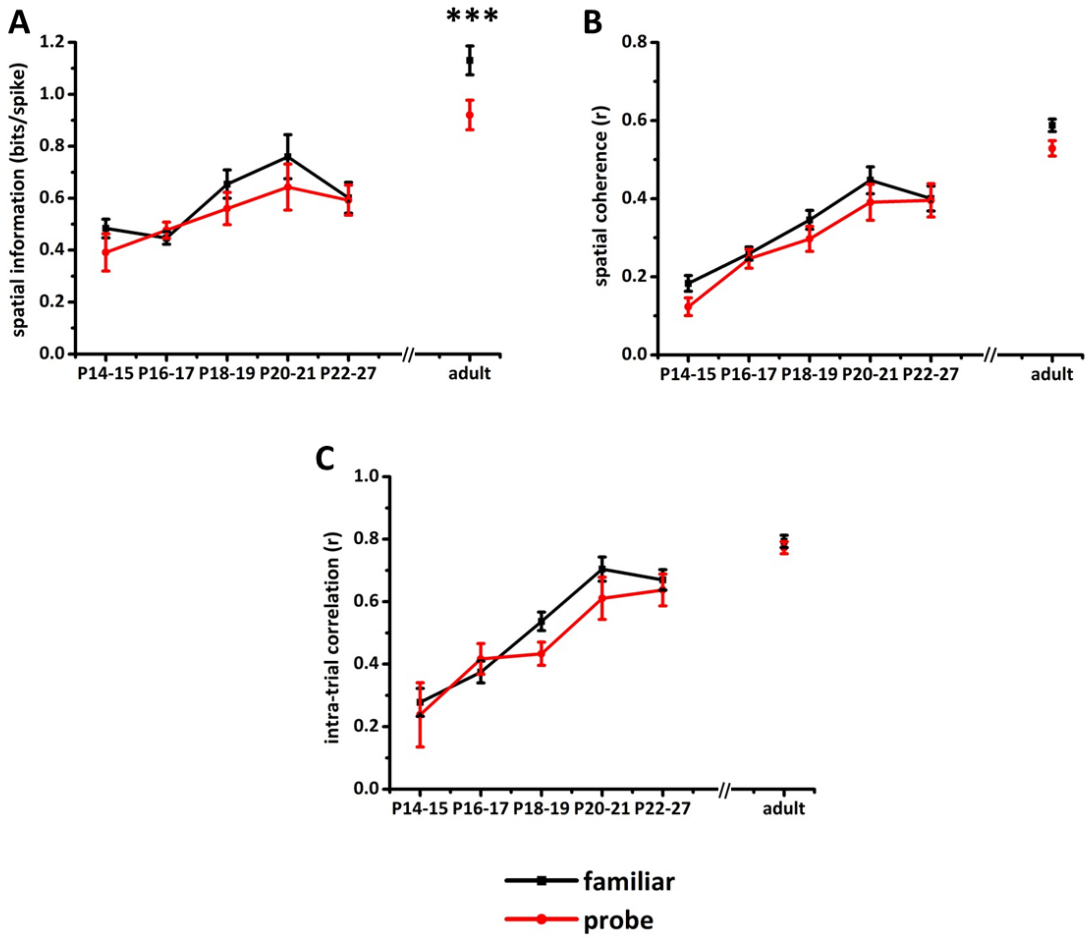


Figure III-27: Quality of the spatial signal of place cells recorded inside the familiar environment (black lines) and under 'new floor' conditions (red lines) between P14-15 and adults. Depicted are spatial information (A, bits/spike), spatial coherence (B, r) and intra-trial correlation (C, r). Values for familiar environment are means across those trials inside the familiar environment before any probe trial was conducted. All values are population means±SEM. ***p<0.001

The population analysis of the quality of the spatial firing of place cells (see Figure III-27) during the 'new floor' probe compared to the one inside the familiar environment yields some surprising results. First all three parameters measuring the quality of the spatial tuning show a

steady increase with age (spatial information: $F_{5,219}$=16.31, p<0.001; spatial coherence: $F_{5,219}$=33.01, p<0.001; intra-trial correlation: $F_{5,218}$=41.95, p<0.001).

Spatial information as well as spatial coherence also show a main effect of environment (spatial information: $F_{1,219}$=5.91, p=0.016; spatial coherence: $F_{1,219}$=6, p=0.015) and the former also shows a significant interaction between age and environment, while for the latter the interaction is non-significant (spatial information: $F_{5,219}$=2.7, p=0.022; spatial coherence: $F_{5,219}$=0.98, p=0.43). The pairwise comparisons for the interaction parameter for spatial information show that only adult controls show a significantly different spatial information content between familiar and probe (p<0.001). All pairwise comparisons for data obtained from rat pups are non-significant (P14-15: p=0.73, P16-17: p=0.58, P18-19: p=0.18, P20-21: p=0.13, P22-27: p=0.75).

Intra-trial correlation neither shows an effect of environment ($F_{1,218}$=0.88, p=0.35) nor an interaction between age and environment ($F_{5,218}$=1.18, p=0.32).

These results are overall quite surprising since they indicate that for rat pups the quality of the spatial tuning is only mildly affected when the floor of a familiar environment is replaced. In contrast, for adult controls place cells contain significantly smaller average spatial information contents during the probe trial.

Stability of spatial firing during 'new floor' probe

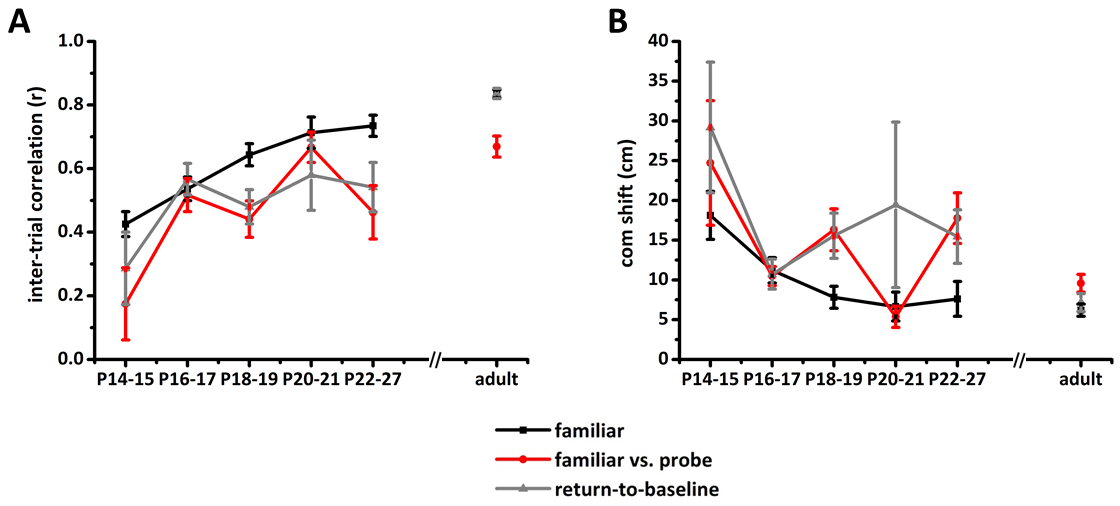


Figure III-28: Stability of the spatial signal of place cells recorded inside the familiar environment (black lines) and under 'new floor' conditions (red lines) between P14-15 and adults. Depicted are inter-trial correlation (A, r) and centre of mass shift (B, cm). The return-to-baseline values (grey lines) indicate the stability of place cells across the two recording trials inside the familiar environment encompassing the probe trial. Values for familiar environment are means across those trials inside the familiar environment before any probe trial was conducted. All values are population means±SEM.

The population analysis of the parameters measuring place cell stability across recording trials for the 'new floor' probe shows no consistent trend during development (see Figure III-28). Just as expected stability of place cells overall increases with age ($F_{5,155}$=18.47, p<0.001), while centre of mass shift decreases in parallel ($F_{5,155}$=7.21, p<0.001).

Both parameters also show a main effect of environment (inter-trial correlation: $F_{2,154}$=8.26, p<0.001; centre of mass shift: $F_{2,154}$=9.55, p<0.001) as well as an interaction between age and environment (inter-trial correlation: $F_{10,310}$=2.96, p=0.001; centre of mass shift: $F_{10,310}$=2.32, p=0.012). This means that i) place cell stability is generally affected (reduced) during the 'new floor' probe and ii) that this effect differs significantly across age. However, from Figure III-28 and Table III-10 it is clear that this effect is due to the rather unsystematic variation of place

cell stability during the probe trial for data recorded from rat pups aged P21 or younger. For adults and post-weanling rat pups (P22-27) the results are rather straight forward. During the probe trial there is a significant reduction/increase in inter-trial correlation/centre of mass shift (see Table III-10), indicating that parts of the place cell population undergoes some form of remapping. For younger animals the results are very difficult to interpret since the stability of place cells during the probe trial 'zigzags' between no effect (P14-15, P16-17 and P20-21) and an effect on place cell stability (P18-19). Moreover the significant differences for the familiar vs. return-to-baseline correlations and centre of mass shifts at P18-19 and P22-27 indicate the above mentioned 'knock-on' effects. This seems to indicate that the probe trial leads to a moderate disruption of the spatial representation of the familiar environment. Note that for P20-21 the return-to baseline values are only based on three units because for this age bin the probe trial often constituted the last trial in the series.

As a conclusion it seems that surprisingly overall place cell stability is only systematically affected during the 'new floor' probe for post-weanling rat pups and adult controls, but not younger rat pups.

Table III-10: Overview of post-hoc pairwise comparisons for the interaction between age and environment for stability measures of place cells for the 'new floor' probe. P-values are indicated for each of the three possible comparisons at each age bin (familiar vs. probe, familiar vs. return-to-baseline and return-to-baseline vs. probe). Red values indicate p-values below the 0.05-significance level.

inter-trial correlation	familiar vs. probe	familiar vs. return-to-baseline	return-to-baseline vs. probe
P14-15	0.15	0.4	0.47
P16-17	0.81	0.5	0.76
P18-19	0.006	0.005	0.7
P20-21	0.3	0.14	0.84
P22-27	0.001	0.005	0.33
adults	<0.001	0.27	<0.001
centre of mass shift	**familiar vs. probe**	**familiar vs. return-to-baseline**	**return-to-baseline vs. probe**
P14-15	0.12	0.03	0.64
P16-17	0.63	0.79	0.81
P18-19	0.001	0.002	0.77
P20-21	0.53	0.048	0.2
P22-27	0.001	0.001	0.7
adults	0.036	0.71	0.07

Active vs. inactive cells during 'new floor' probe

The numbers of place cells that either are exclusively active during the 'new floor' probe or cease firing during the probe trial potentially indicate some form of developmental change (see Table III-11). In pups it is rather rare for cells to show probe trial specific activity/inactivity. It also does not seem there is a systematic change of those numbers across the different age bins containing rat pup data. For adults the numbers are generally higher which seems to further support the stronger effect of this probe trial type on adult animals. Note that the low number of cells in either category for P14-15 is due to a large number of cells that show an inconsistent firing pattern across the familiar trials of the series.

Table III-11: Overview of place cell numbers that are active in both environments and those that are either exclusively active during probe ('probe only') or familiar ('familiar only') trials for the 'new floor' probe. Given are absolute numbers as well as proportion from the place cell population defined by their overall spatiality in a probe series in parentheses. Note that cells showing an inconsistent firing pattern across all familiar trials are excluded from this analysis.

	# both environments (%)	# probe only (%)	# familiar only (%)
P14-15	7 (25%)	0 (0%)	2 (7%)
P16-17	42 (91%)	1 (2%)	1 (2%)
P18-19	36 (90%)	0 (0%)	0 (0%)
P20-21	16 (89%)	0 (0%)	1 (6%)
P22-27	26 (93%)	1 (4%)	0 (0%)
adult	93 (83%)	4 (4%)	9 (8%)

III.4.2.3 Effects of replacing the floor and walls of the familiar environment ('new floor & new walls')

The previous two sections described the effects of replacing individual parts (floor and walls respectively) of the recording environment. This section describes the effects of replacing both parts for visual replicas in one single probe trial.

Table III-12: Overview of recorded cell numbers for the 'new floor & new walls' probe across development. Given are numbers of complex spike cells (left column), place cells as defined for the analysis of within-trial measures (middle column) and stability filtered place cells for the analysis of the across-trial measures (right column).

	# complex spike cells	# place cells (according to 'spatiality')	# place cells (stability filtered)
P14-15	no cells	no cells	no cells
P16-17	117	49	34
P18-19	103	42	26
P20-21	62	28	20
P22-27	60	31	24
adult	124	107	89

Table III-12 gives an overview of recorded cell numbers at each respective age bin. Again numbers of complex spike cells, place cells defined by their overall spatiality across the whole trial series and stability filtered place cells are given.

Figure III-29 shows representative rate maps of place cells recorded for this probe trial type for each age bin (note that there is no data for P14-15). It is clear that especially for young animals (< P22) this type of probe has a profound effect on the location-specific firing of place cells. In these animals replacing the floor and walls of a familiar environment leads to remapping for

most of the place cell population. During the probe trial place fields move to a new location (e.g. cell 1 and 2 at P16-17; cell 1 and 2 at P18-19; cell 2 and 3 at P20-21), cease firing (cell 3 at P18-19) or lose spatial specificity of firing (cell 3 at P16-17; cell 1 at P20-21). For post-weanling pups and adult controls the responses of place cells during the probe are rather mixed. Some cells (e.g. cell 2 and 3 at P22-27; cell 2 and 3 for adults) more or less maintain their firing field from the familiar trials, while a subpopulation of place cells does show remapping during the probe trial (cell 1 at P22-27; cell 1 for adults). It is noteworthy that, especially for adults, place cells that do not show a stable field during the probe typically remap by either shifting their fields or cease/start activity, but never lose spatial specificity of firing.

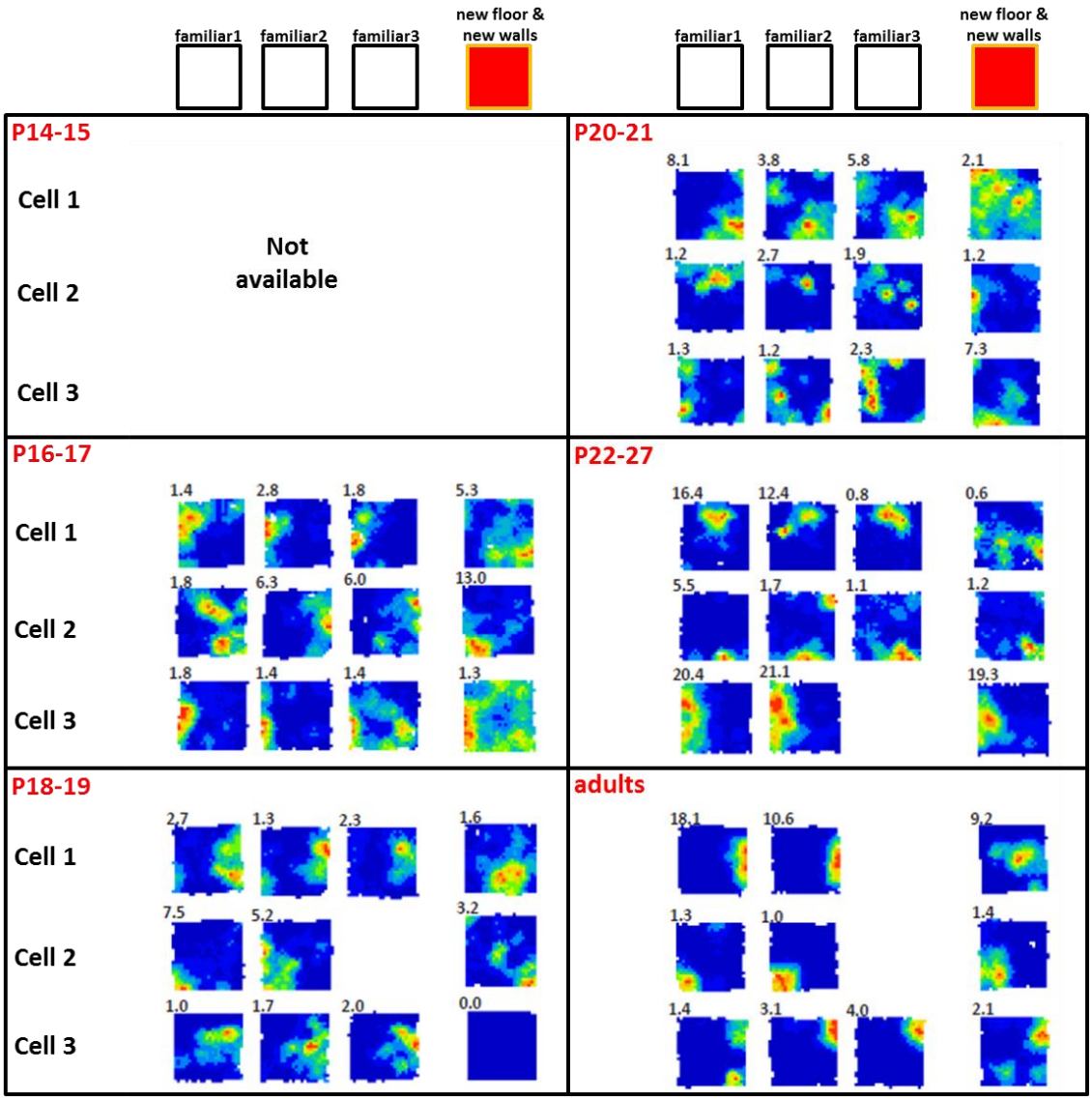


Figure III-29: Representative rate maps of place cells recorded inside the familiar environment and under 'new floor & new walls' conditions across development (P16-17 – adults). For each age bin (black squares) 3 representative examples are shown across familiar trials ('familiar1-3') run before the probe trial and the probe trial itself ('new floor & new walls'). Because this probe trial type usually constituted the last trial in a probe series no familiar trials after the probe are shown. All example cells shown have average spatial information scores across 'familiar1-3' in the range of age mean±standard deviation. Top row indicates schematic of trial sequence. Rate maps are false colour auto-scaled heat maps with progressively warmer colours indicating progressively higher firing rates. Peak firing rates (Hz) are indicated at top left corner of each rate map. P(x)-(x) indicates age bin. Note that there is no data for P14-15.

Population analysis of place cell properties for the 'new floor & new walls' probe

Quality of spatial firing during 'new floor & new walls' probe

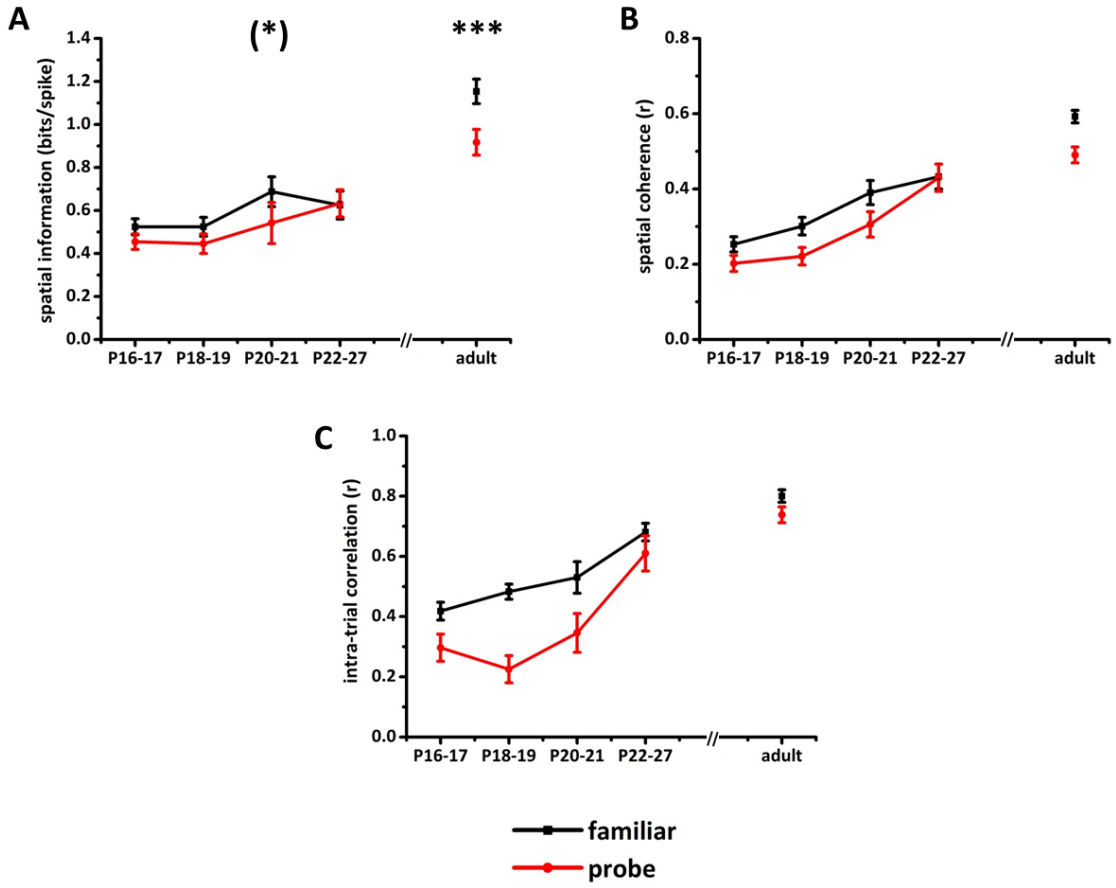


Figure III-30: Quality of the spatial signal of place cells recorded inside the familiar environment (black lines) and under 'new floor & new walls' conditions (red lines) between P16-17 and adults. Depicted are spatial information (A, bits/spike), spatial coherence (B, r) and intra-trial correlation (C, r). Values for familiar environment are means across those trials inside the familiar environment before any probe trial was conducted. All values are population means±SEM. Note that there is no data available for P14-15. (*)p=0.05, ***p<0.001

The population analysis of the quality of the spatial tuning of place cells during the 'new floor & new walls' probe shows comparable results to the ones from the 'new floor' probe with the

exception of intra-trial correlation (compare Figure III-27 and Figure III-30). All parameters increase significantly with age (spatial information: $F_{4,210}$=20.72, p<0.001; spatial coherence: $F_{4,210}$=42.18, p<0.001; intra-trial correlation: $F_{4,209}$=68.82, p<0.001).

The quality of place cell firing (see Figure III-30) is significantly affected during 'new floor & new walls' probe trials (main effect of environment: spatial information: $F_{1,210}$=12.16, p=0.001; spatial coherence: $F_{1,210}$=20.07, p<0.001; intra-trial correlation: $F_{1,209}$=18.72, p<0.001). Moreover, there is a significant interaction between age and environment for spatial information ($F_{4,210}$=2.92, p=0.022), but not for the other two parameters (spatial coherence: $F_{4,210}$=2.29, p=0.06; intra-trial correlation: $F_{4,209}$=0.71, p=0.56). The pairwise comparisons reveal that spatial information only differs significantly between familiar and probe trials for adult controls (p<0.001). All other comparisons are non-significant (P16-17: p=0.17; P18-19: p=0.39; P20-21: p=0.051; P22-27: p=0.84), although it is noteworthy that for data recorded at P20-21 the significance level is just at the 0.05-level.

These results are thus similar to the ones obtained in the 'new floor' probe (see Figure III-27). Only adult place cells seem to show a strong effect on the quality of the spatial tuning during the probe trial. Surprisingly intra-trial correlation is significantly affected during the probe trial across all age bins, highlighting that replacing both floor and walls of a familiar environment has a more wide-spread effect on the quality of place cell firing compared to replacing the floor (or the walls) alone.

Stability of spatial firing during 'new floor & new walls' probe

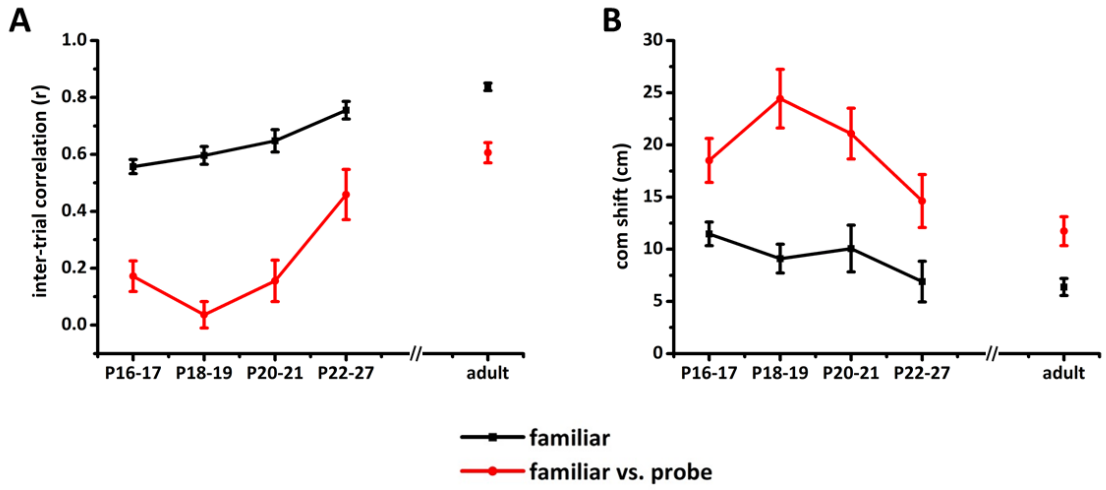


Figure III-31: Stability of the spatial signal of place cells recorded inside the familiar environment (black lines) and under 'new floor & new walls' conditions (red lines) between P16-17 and adults. Depicted are inter-trial correlation (A, r) and centre of mass shift (B, cm). Note that no return-to-baseline correlation is available for this probe trial type because in most cases it was the last trial of a series. All values are population means±SEM. Note that no data is available for P14-15.

In contrast to the 'new floor' probe results (see Figure III-28), the population-wide analysis of place cell stability yields very consistent results for the 'new floor & new walls' probe trial (see Figure III-31). Both parameters either increase (inter-trial correlation) or decrease (centre of mass shift) significantly with age (inter-trial correlation: $F_{4,168}$=38.85, p<0.001; centre of mass shift: $F_{4,168}$=8.1, p<0.001).

Additionally, both measures show a main effect of recording environment (inter-trial correlation: $F_{1,168}$=139.06, p<0.001; centre of mass shift: $F_{1,168}$=60.1, p<0.001). Inter-trial correlation does not show an interaction between age and environment (inter-trial correlation: $F_{4,168}$=0.97, p=0.43), while for centre of mass shift the p-value for the interaction is exactly at the 0.05-significance level (centre of mass shift: $F_{4,168}$=2.43, p=0.05). The pairwise comparisons

for the latter show significantly higher centre of mass shifts during the probe trial for all age bins (P16-17: $p=0.005$; P18-19: $p<0.001$; P20-21: $p=0.001$; P22-27: $p=0.002$; adults: $p=0.001$). Note that because this type of probe trial was in most cases the last recording trial in a given series, there is no return-to-baseline correlation.

These results show that removing the local olfactory and tactile cues of a familiar environment has a quite profound effect on place cell stability at all age bins, in that more or less strong remapping is induced at all ages.

Active vs. inactive cells during 'new floor & new walls' probe

Table III-13: Overview of place cell numbers that are active in both environments and those that are either exclusively active during probe ('probe only') or familiar ('familiar only') trials for the 'new floor & new walls' probe. Given are absolute numbers as well as proportion from the place cell population defined by their overall spatiality in a probe series in parentheses. Note that cells showing an inconsistent firing pattern across all familiar trials are excluded from this analysis.

	# both environments (%)	# probe only (%)	# familiar only (%)
P14-15	no cells	no cells	no cells
P16-17	45 (92%)	1 (2%)	1 (2%)
P18-19	32 (76%)	1 (2%)	6 (14%)
P20-21	24 (86%)	1 (4%)	1 (4%)
P22-27	28 (90%)	0 (0%)	0 (0%)
adult	91 (85%)	4 (4%)	6 (6%)

The amount and percentage of place cells showing a probe trial specific activity or ceasing of activity is somewhat similar to the new floor probe (see Table III-13, compare to Table III-11). While the numbers are pretty constant for place cells recorded from rat pups at any age (except 'familiar only' cells at P18-19), adult controls show slightly higher absolute numbers and percentages.

III.4.2.4 Summary of results from manipulations of the local olfactory/tactile cues inside a familiar environment

Replacing one or several parts of a familiar environment by visual replicas has very different effects on place cell quality and stability (see Figure III-24, Figure III-25, Figure III-27, Figure III-28, Figure III-30, Figure III-31). Replacing only the walls has only little effect on place cell quality and stability at any age bin (see Figure III-24 and Figure III-25), and though there is a reduction in place cell stability at some ages, this does not reach statistical significance. These results indicate that local cues located on the walls are either not essential for place cell firing or that the remaining cues are enough to largely re-activate the place cell representation of the familiar environment.

When the floor is replaced by a visual replica, place cell stability is consistently reduced in post-weanling animals and adult controls (see Figure III-28). Interestingly only the latter group also shows a clear reduction in place cell quality during this probe trial (see Figure III-27). When both floor and walls are replaced in one single probe trial, the response of place cells is rather clear cut. At all ages there is a reduction in place cell stability (see Figure III-31). For this probe again the quality of place cell firing is only strongly affected for adult controls (see Figure III-30), except intra-trial correlation which is strongly affected in rat pups as well as in adult controls (see Figure III-30C).

When comparing the ‘new floor‘ and ‘new floor & walls‘ probes, it seems that the response of place cells in adult controls is very similar in both, i.e. a reduction in quality (see Figure III-27 and Figure III-30) and in stability (see Figure III-28 and Figure III-31). Furthermore, the responses of place cells in post-weanling animals seem to be similar to those seen in adults. Surprisingly, for pre-weanling rat pups there are different responses to these two probe trial types (strong response to changing floor and walls, no or variable response to changing only

walls), perhaps indicating differences in the functional significance of local cues inside an environment at pre-weanling ages.

III.4.3 Removal of visual cues in a familiar environment ('familiar dark')

The previous sections described the responses of place cells during probe trials when parts of a familiar environment were replaced for visual replicas. This section deals with place cell responses during an absence of any visual cues (i.e. lights off) inside the familiar environment.

For an overview of the numbers of cells recorded at each age bin for the 'familiar dark' probe see Table III-14.

Table III-14: Overview of recorded cell numbers in the 'familiar dark' probe across development. Given are numbers of complex spike cells (left column), place cells as defined for the analysis of within-trial measures (middle column) and stability filtered place cells for the analysis of the across-trial measures (right column).

	# complex spike cells	# place cells (according to 'spatiality')	# place cells (stability filtered)
P14-15	50	25	8
P16-17	92	63	28
P18-19	105	62	25
P20-21	123	61	37
P22-27	33	12	10
adult	141	113	88

When looking at some representative example rate maps of place cells recorded under familiar conditions and during the probe trial, it becomes obvious that place cells show a rather mixed response during the absence of visual cues (see Figure III-32). On the one hand, there is a subset of cells at each age bin that keep their firing fields from the light recording (cell 1 at each age bin). On the other, some cells do show a shift of their firing fields (cell 2 at P14-15,

cell 3 at P16-17, cell 3 at P20-21), cease activity in darkness (cell 2 at P20-21, cell 3 at adults) or lose their spatial specificity (cell 2 at P18-19). Occasionally place cells seem to keep their field location from the familiar trials, but show an increase in field size (cell 3 at P14-15, cell 2 at P16-17, cell 3 at P18-19, cells 2 and 3 at P22-27, cell 2 at adults). Note that some cells show a 'knock-on' effect. Cell 3 at P14-15, cell 2 at P16-17, cell 3 at P20-21, or cell 3 at P22-27 show some form of change of their firing fields during the familiar trial after the probe in comparison to the familiar trials preceding the probe.

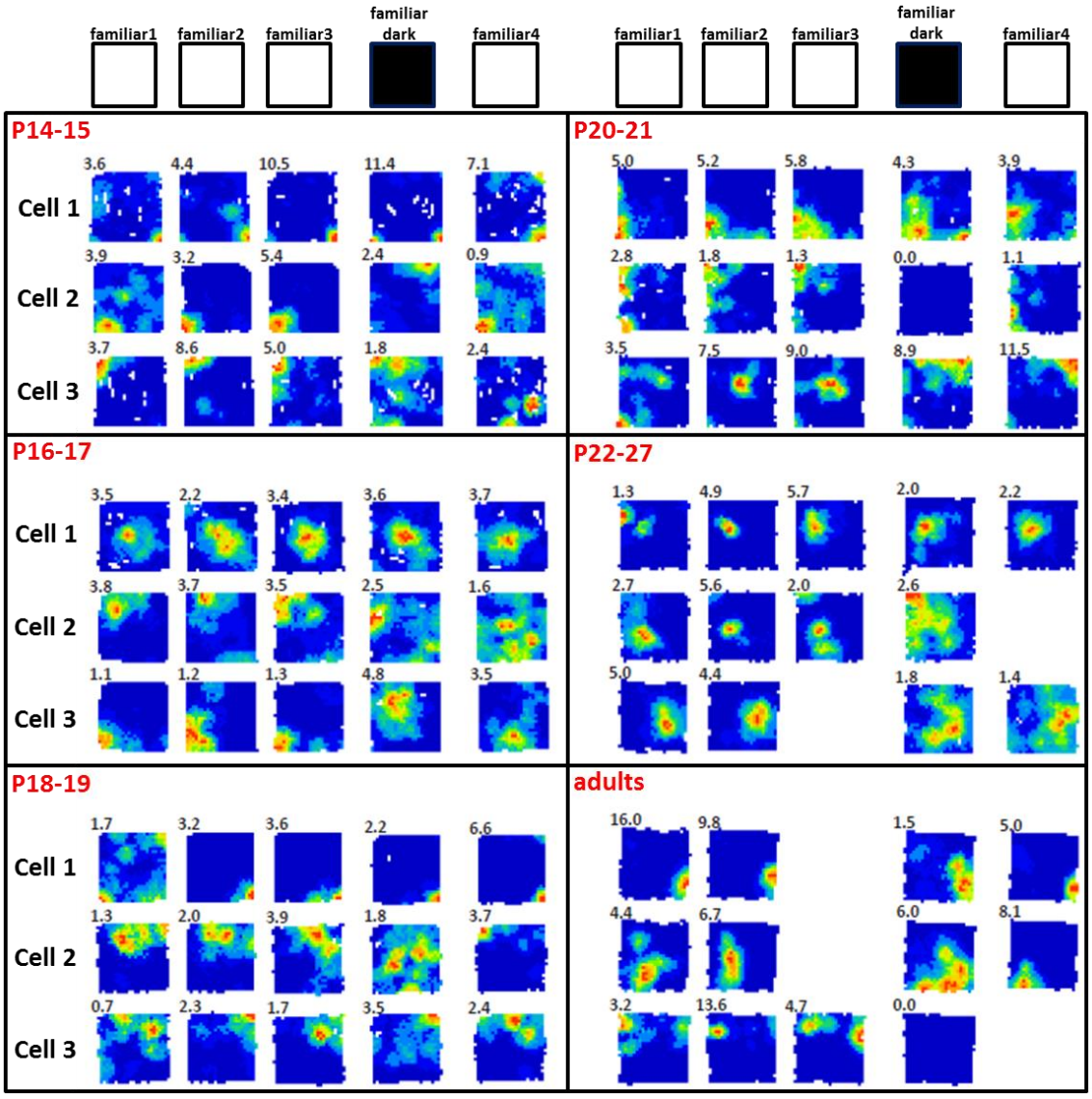


Figure III-32: Representative rate maps of place cells recorded inside the familiar environment and under 'familiar dark' conditions across development (P14-15 – adults). For each age bin (black squares) 3 representative examples are shown across familiar trials ('familiar1-3') run before the probe trial, the probe trial itself ('familiar dark') and a following familiar trial ('familiar4'). All example cells shown have average spatial information scores across 'familiar1-3' in the range of age mean±standard deviation. Top row indicates schematic of trial sequence. Rate maps are false colour auto-scaled heat maps with progressively warmer colours indicating progressively higher firing rates. Peak firing rates (Hz) are indicated at top left corner of each rate map. P(x)-(x) indicates age bin.

Population analysis of place cell properties for the 'familiar dark' probe

Quality of spatial firing during 'familiar dark' probe

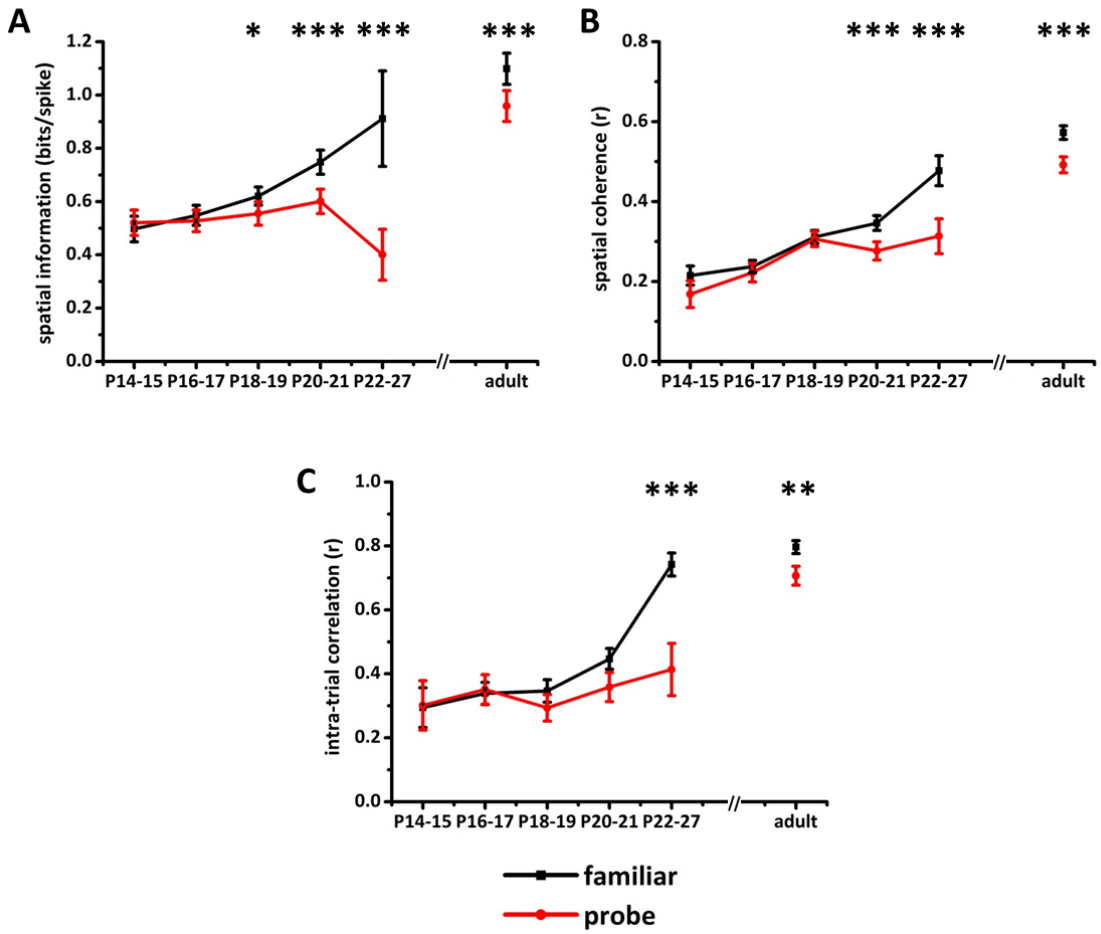


Figure III-33: Quality of the spatial signal of place cells recorded inside the familiar environment (black lines) and under 'familiar dark' conditions (red lines) between P14-15 and adults. Depicted are spatial information (A, bits/spike), spatial coherence (B, r) and intra-trial correlation (C, r). Values for familiar environment are means across those trials inside the familiar environment before any probe trial was conducted. All values are population means±SEM. *p<0.05, **p<0.01, ***p<0.001

The population analysis of the change in the quality of the spatial firing of place cells recorded under 'familiar dark' conditions reveals some interesting age-dependent effects (see Figure III-

33). Just as for all previously described probe trials all parameters show a significant increase with age (spatial information: $F_{5,267}$=15.84, p<0.001; spatial coherence: $F_{5,267}$=45.9, p<0.001; intra-trial correlation: $F_{5,267}$=55.8, p<0.001).

All three measures of the quality of the spatial tuning reveal a main effect of recording environment (spatial information: $F_{1,267}$=30.09, p<0.001; spatial coherence: $F_{1,267}$=26.91, p<0.001; intra-trial correlation: $F_{1,267}$=16.58, p<0.001) as well as a significant interaction between age and environment (spatial information: $F_{5,267}$=3.29, p=0.007; spatial coherence: $F_{5,267}$=3.79, p=0.002; intra-trial correlation: $F_{5,267}$=3.36, p=0.006). The pairwise comparisons for the interaction parameter confirm the result that is already obvious from Figure III-33. Only data recorded from post-weanling pups and adult controls shows a significant difference across all three measures between familiar and 'familiar dark' trials (see Table III-15). On the other hand, none of the pairwise comparisons differ significantly for data obtained between P14-17 (see Table III-15). For P18-19 and P20-21 the results are mixed across the three measures with some showing significant differences while others do not (Table III-15).

These results are very interesting since they seem to indicate an increasing influence of visual cues for the quality of the location specific firing of place cells throughout development.

Table III-15: Overview of results from pairwise comparisons for the interaction between age and environment for the quality of the spatial signal during the 'familiar dark' probe. p-values are indicated for spatial information, spatial coherence and intra-trial correlation. Red values are below 0.05-significance level.

	spatial information	spatial coherence	intra-trial correlation
P14-15	0.86	0.46	0.91
P16-17	0.39	0.42	0.77
P18-19	0.04	0.7	0.36
P20-21	<0.001	0.001	0.12
P22-27	<0.001	0.001	<0.001
adults	<0.001	0.001	0.001

Stability of spatial firing during 'familiar dark' probe

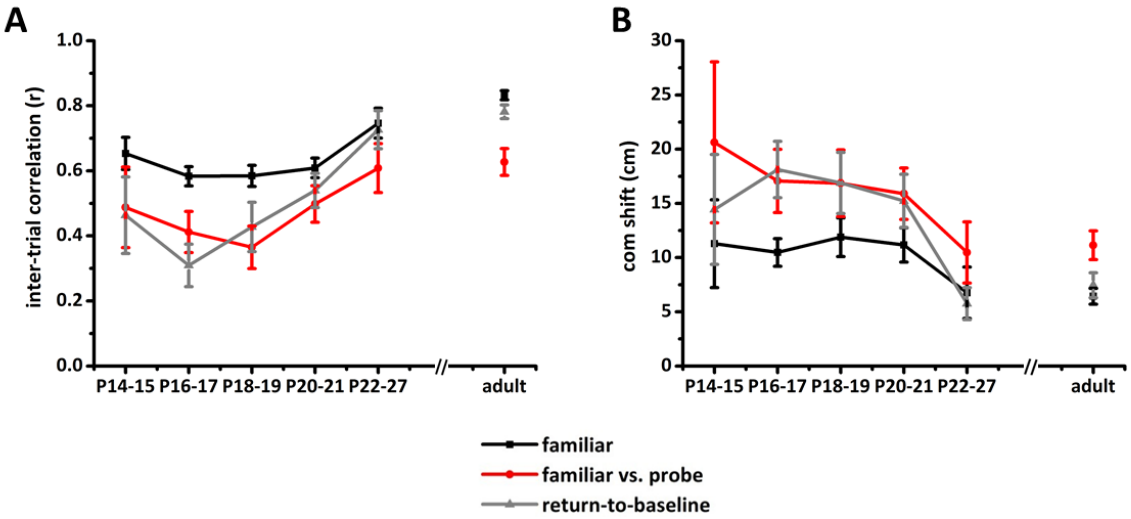


Figure III-34: Stability of the spatial signal of place cells recorded inside the familiar environment (black lines) and under 'familiar dark' conditions (red lines) between P14-15 and adults. Depicted are inter-trial correlation (A, r) and centre of mass shift (B, cm). The return-to-baseline values (grey lines) indicate the stability of place cells across the two recording trials inside the familiar environment encompassing the probe trial. Values for familiar environment are means across those trials inside the familiar environment before any probe trial was conducted. All values are population means±SEM.

The population-wide analysis of place cell stability during the 'familiar dark' probe shows a rather consistent effect across all ages (see Figure III-34). Both measures increase/decrease significantly with age (inter-trial correlation: $F_{5,167}=18.35$, $p<0.001$; centre of mass shift: $F_{5,167}=5.06$, $p<0.001$).

The difference in stability between familiar and probe trials is consistent across all age bins (see Figure III-34). At all ages a significant, moderately reduced familiar vs. probe correlation in comparison to the average familiar correlation is present (main effect of environment: $F_{2,166}=11.85$, $p<0.001$), as well as larger centre of mass shifts during the probe trial (main effect of environment: $F_{2,166}=7.94$, $p=0.001$). Both measures do not show a significant interaction

between age and environment (inter-trial correlation: $F_{10,334}$=1.54, p=0.12; centre of mass shift: $F_{10,334}$=0.97, p=0.47). The reduced values of the return-to-baseline correlation for all ages until P21 seem to indicate the presence of 'knock-on' effects from the probe trial, which can also be seen with some of the representative rate maps at these age bins (see Figure III-32).

Compared to the analysis of the quality of the spatial firing of place cells these results are quite interesting, since they indicate that visual cues do have an impact on place cell stability as early as P14-15, although the quality is unaffected at these ages. P14-15 typically denotes the time point of eye opening in rat pups. Another interesting effect is the fact that the absence of visual cues inside a familiar environment seems to have a weaker effect on place cell stability compared to replacing the floor and walls together, especially for pre-weanling rat pups (compare Figure III-31 and Figure III-34). This could indicate a weaker control of visual cues than local olfactory and tactile ones on place cell stability in these animals.

Active vs. inactive cells during 'familiar dark' probe

The number of place cells which are exclusively active in either familiar or probe trial seems to increase slightly between pups and adults with the exception of data recorded at P14-15 (see Table III-16), but overall only constitute a rather small fraction of the whole recorded cell population (< 10-15%).

Table III-16: Overview of place cell numbers that are active in both environments and those that are either exclusively active during probe ('probe only') or familiar ('familiar only') trials for the 'familiar dark' probe. Given are absolute numbers as well as proportion from the place cell population defined by their overall spatiality in a probe series in parentheses. Note that cells showing an inconsistent firing pattern across all familiar trials are excluded from this analysis.

	# both environments (%)	# probe only (%)	# familiar only (%)
P14-15	17 (68%)	1 (4%)	3 (12%)
P16-17	51 (81%)	3 (5%)	1 (2%)
P18-19	55 (89%)	1 (2%)	1 (2%)
P20-21	52 (85%)	0 (0%)	3 (5%)
P22-27	11 (92%)	0 (0%)	1 (8%)
Adult	87 (77%)	6 (5%)	8 (7%)

III.4.4 Recordings in a completely novel environment

III.4.4.1 Introduction

So far all the discussed probe trials dealt with manipulations of parts of the sensory cues in a highly familiar environment. In adult rats the standard protocol for investigating remapping of place fields is typically recording place cells in a novel environment. A novel environment is usually either located elsewhere to the familiar one (i.e. different room or different location in the same room), made of different material or shape or all of the above. Recording the responses of place cells of developing rat pups in such a novel environment constitutes an important control condition. This is because firstly the response of adult place cells to such a manipulation is very well characterised and secondly it will thus show if rat pups are indeed able to distinguish between two distinct environments. In case e.g. recording in a novel environment will not lead to a more or less complete remapping of the place cell representation in rat pups, this would cast some doubt on the general ability of these animals to detect drastic changes in the recording environment. Two types of novel environment probe trials were conducted for this thesis: recordings in a novel environment under light and under dark conditions. By comparing the quality of spatial firing in light and dark conditions, a better assessment of the necessity of visual cues in setting up a new place cell representation will be possible. For rat pups these environments were located in a separate location to the familiar environment, but inside the same experimental room. The location of the novel environments was surrounded by curtains and both were of the same shape (square) as the familiar environment, but consisted of different floor and wall materials (see section II.4.1.3, p.

109). For adults a similar setup was used with the main difference being the fact that the novel environments were not surrounded by curtains.

III.4.4.2 Recordings in a novel environment under light conditions ('novel light')

Table III-17: Overview of recorded cell numbers in the 'novel light' probe across development. Given are numbers of complex spike cells (left column), place cells as defined for the analysis of within-trial measures (middle column) and stability filtered place cells for the analysis of the across-trial measures (right column).

	# complex spike cells	# place cells (according to 'spatiality')	# place cells (stability filtered)
P14-15	no cells	no cells	no cells
P16-17	23	20	11
P18-19	30	16	8
P20-21	47	22	14
P22-27	9	6	4
adult	133	112	73

Table III-17 gives the numbers of place cells recorded in the novel environment under light conditions for each age bin. Note that for P22-27 only a rather small number of place cells from only one animal was recorded (n=6 and 4; see Table III-17). This is why for this age bin some example rate maps are presented, but no quantitative analysis of the respective place cell properties is performed (see Figure III-35, Figure III-36 and Figure III-37). The data for P16-17 was also recorded from only one animal, but since this dataset is more than twice as large, the properties of place cells were nonetheless quantified statistically for this age bin.

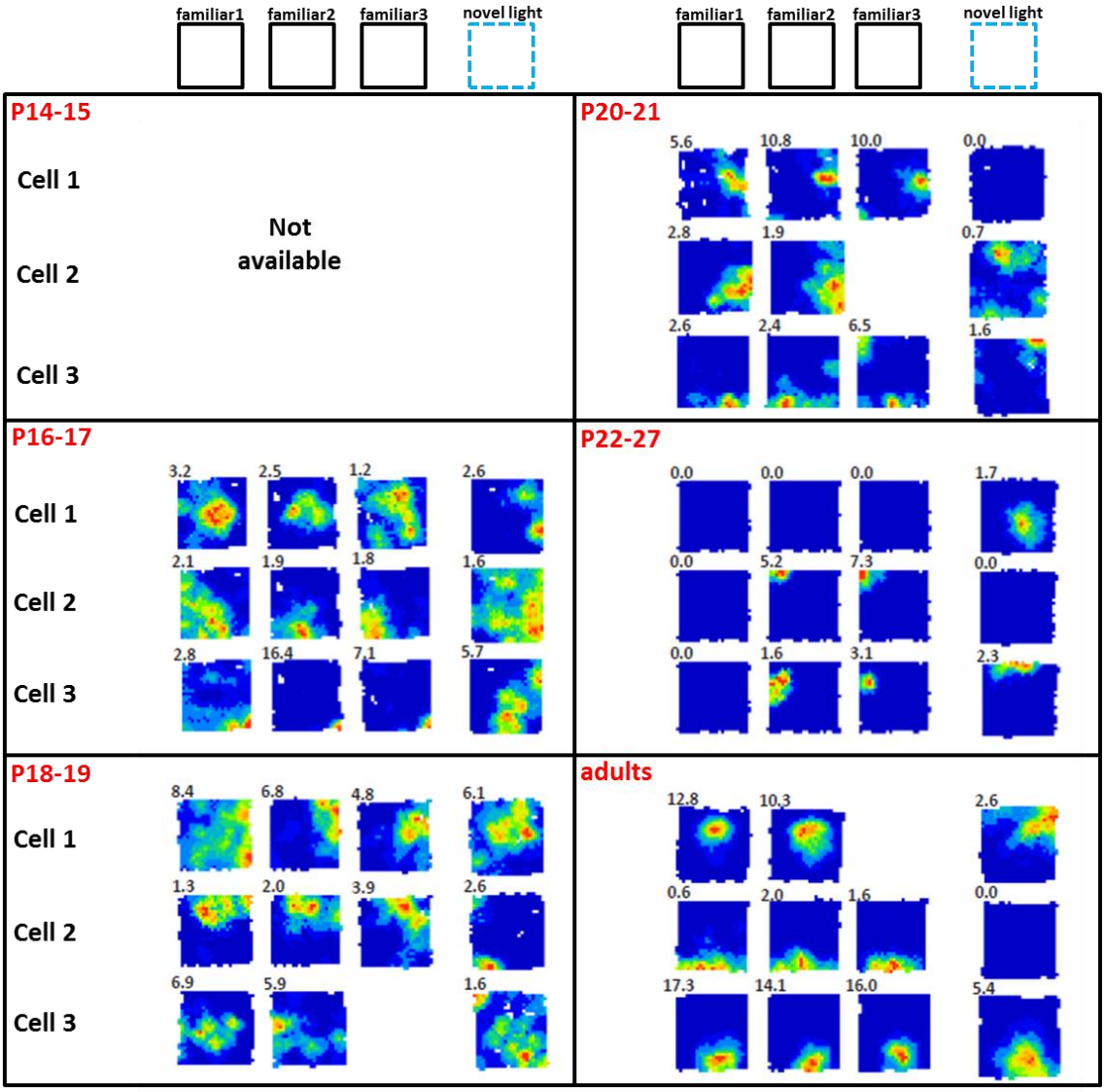


Figure III-35: Representative rate maps of place cells recorded inside the familiar environment and under 'novel light' conditions across development (P16-17 – adults). For each age bin (black squares) 3 representative examples are shown across familiar trials ('familiar1-3') run before the probe trial and the probe trial itself ('novel light'). Because this probe trial type usually constituted the last trial in a probe series no familiar trials after the probe are shown. All example cells shown have average spatial information scores across 'familiar1-3' in the range of age mean±standard deviation. Top row indicates schematic of trial sequence. Rate maps are false colour auto-scaled heat maps with progressively warmer colours indicating progressively higher firing rates. Peak firing rates (Hz) are indicated at top left corner of each rate map. P(x)-(x) indicates age bin. Note that there is no data for P14-15.

When comparing the responses of place cells between the familiar and novel environment, the results are very clear cut for all age bins containing data from rat pups (see Figure III-35). Place cells at each of those age bins show a typical remapping response between the familiar and novel environment. This includes place fields moving to an unpredictable new location (cell 1 and 3 at P16-17, cell 2 and 3 P18-19, cell 2 and 3 at P20-21, cell 3 at P22-27), ceasing of unit activity (cell 1 at P20-21, cell 2 at P22-27) and the loss of their spatial specificity (cell 2 at P16-17). For adult controls the responses are somewhat more ambiguous. On the one hand, a lot of place cells either cease activity (cell 2) or start becoming active (no example shown) inside the novel environment. A smaller subset shows remapping of the firing field (cell 1), but another subset actually does not show remapping at all (cell 3) and shows the same firing field location as inside the familiar environment.

Population analysis of place cell properties for the 'novel light' probe

Quality of spatial firing during 'novel light' probe

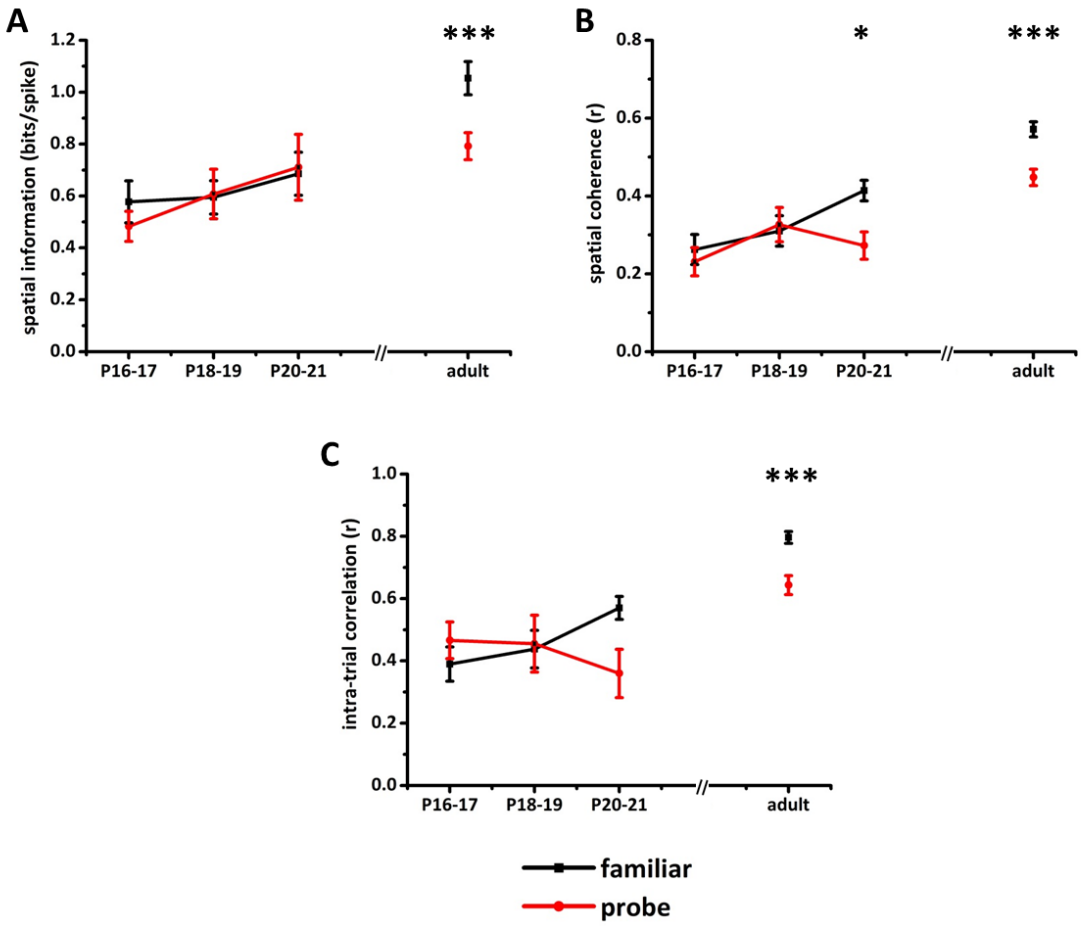


Figure III-36: Quality of the spatial signal of place cells recorded inside the familiar environment (black lines) and under 'novel light' conditions (red lines) between P16-17 and adults. Depicted are spatial information (A, bits/spike), spatial coherence (B, r) and intra-trial correlation (C, r). Values for familiar environment are means across those trials inside the familiar environment before any probe trial was conducted. All values are population means±SEM. Note that there is no data for P14-15 and P22-27. *p<0.05, ***p<0.001

The population-wide analysis of the quality of the spatial firing during the 'novel light' probe shows some interesting interactions between age and recording environment (see Figure III-

36). All three parameters show a significant increase with age (spatial information: $F_{3,103}$=2.88, p=0.04; spatial coherence: $F_{3,103}$=15.44, p<0.001; intra-trial correlation: $F_{3,103}$=22.92, p<0.001).

While spatial coherence shows a main effect of recording environment ($F_{1,103}$=8.83, p=0.004), this effect is not present for spatial information and intra-trial correlation (spatial information: $F_{1,103}$=0.77, p=0.38; intra-trial correlation: $F_{1,103}$=1.19, p=0.28). In contrast the interaction between age and environment is significant for all three measures (spatial information: $F_{3,103}$=4, p=0.01; spatial coherence: $F_{3,103}$=2.97, p=0.035; intra-trial correlation: $F_{3,103}$=5.73, p=0.001). The significant interaction is most probably due to the differences between familiar and probe trials for the adult dataset. The pairwise comparisons only show significant differences across all measures for this dataset (spatial information: p<0.001; spatial coherence: p<0.001; intra-trial correlation: p<0.001). For data recorded from rat pups only spatial coherence reveals a significant difference in the pairwise comparisons for data obtained at P20-21 (p=0.02). All other comparisons are non-significant (spatial information: P16-17: p=0.43, P18-18: p=0.61, P20-21: p=0.35; spatial coherence: P16-17: p=0.7, P18-19: p=1; intra-trial correlation: P16-17: p=0.31, P18-19: p=0.41, P20-21: p=0.2).

These are again very interesting results since they highlight a different effect of novelty on the quality of the spatial tuning of place cells throughout development. Before P20 novelty does not affect this tuning at all in contrast to adult control animals which show a clear effect for all three measures. Unfortunately there is not enough data for post-weanling rat pups to see whether there is a similar effect to adults or whether the effect is still absent. The mean values for the six units recorded at this age bin however seem to indicate an adult-like reduction in the spatial tuning during the probe trial (spatial information: familiar: 1.62±0.3, probe: 0.85±0.2; spatial coherence: familiar: 0.55±0.05, probe: 0.38±0.01; intra-trial correlation: familiar: 0.78±0.04, probe: 0.66±0.16).

Stability of spatial firing during 'novel light' probe

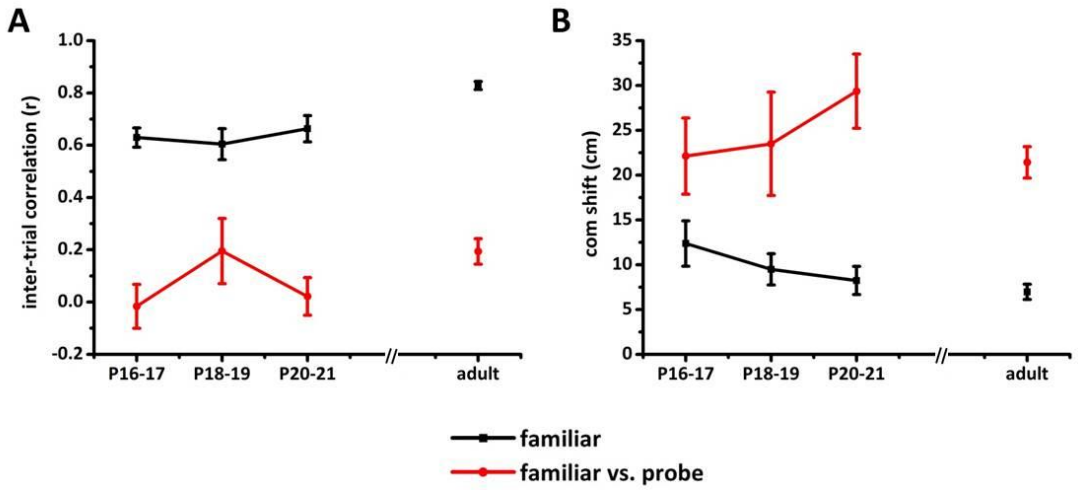


Figure III-37: Stability of the spatial signal of place cells recorded inside the familiar environment (black lines) and under 'novel light' conditions (red lines) between P16-17 and adults. Depicted are inter-trial correlation (A, r) and centre of mass shift (B, cm). Note that no return-to-baseline correlation is available for this probe trial type because in most cases it was the last trial of a series. All values are population means±SEM. Note that no data is available for P14-15 and P22-27.

The results for the stability of place cells between the familiar and novel environment in light conditions are quite clear (see Figure III-37). As far as the effects of age are concerned only inter-trial correlation shows a significant effect of this factor ($F_{3,75}$=8.11, p<0.001), while for the centre of mass shifts there is no main effect of age ($F_{3,75}$=1.58, p=0.2).

Both inter-trial correlation and centre of mass shift show a main effect of environment (inter-trial correlation: $F_{1,75}$=113.96, p<0.001; centre of mass shift: $F_{1,75}$=46.81, p<0.001) and no significant interaction between age and environment (inter-trial correlation: $F_{3,75}$=2.33, p=0.08; centre of mass shift: $F_{3,75}$=0.93, p=0.43). This means that regardless of age, place cell stability is greatly reduced between familiar and 'novel light' trials. In other words, even in very young animals place cells remap when recorded inside a novel environment. Note however that the

familiar vs. probe correlation of ca. 0.2 in adult animals shows that a fraction of the cell population keeps the location of their firing fields from the familiar recordings, even inside the novel environment. This 'residual' stability may be an artefact of a difference in experimental protocol between adults and pups: the adult novel environment did not have black screening curtains, so extra-maze cues were shared between the novel and familiar environments. As already mentioned above, data recorded between P22-27 was not included in the population-wide statistical analysis, because of the overall low numbers of cells for this age bin. Nonetheless, place cells recorded at this age show the principle result: The average familiar to novel environment correlation is (mean±SEM) r=0.04±0.02 (vs. r=0.79±0.04 average familiar inter-trial correlation) and the average centre of mass shift from familiar to probe trials is 21.16±2.95 cm (vs. 4.13±1.63 cm average familiar centre of mass shift).

These results clearly demonstrate that a new place cell representation is formed in a novel environment, regardless of the age of the animals.

Active vs. inactive cells during 'novel light' probe

Table III-18: Overview of place cell numbers that are active in both environments and those that are either exclusively active during probe ('probe only') or familiar ('familiar only') trials for the 'novel light' probe. Given are absolute numbers as well as proportion from the place cell population defined by their overall spatiality in a probe series in parentheses. Note that cells showing an inconsistent firing pattern across all familiar trials are excluded from this analysis.

	# both environments (%)	# probe only (%)	# familiar only (%)
P14-15	no cells	no cells	no cells
P16-17	19 (95%)	1(5%)	0 (0%)
P18-19	12 (75%)	0 (0%)	2 (13%)
P20-21	18 (82%)	0 (0%)	4 (18%)
P22-27	2 (33%)	2 (33%)	2 (33%)
adult	65 (58%)	16 (14%)	20 (18%)

The number of place cells being exclusively active in either the familiar or the novel environment in adult controls show that ca. 32% (36/112) of the whole recorded place cell population is only active in one of the two environments (see Table III-18). The numbers for the pup datasets seem to generally be lower (except at P22-27) and actually seem to increase across development (see Table III-18).

III.4.4.3 Recordings in a novel environment under dark conditions ('novel dark')

To exclude the possibility that the ability of rat pups to detect a novel environment is solely based on visual cues/input, some probe trials were run in a novel environment in dark conditions. Since the 'familiar dark' probe was conducted in an otherwise highly familiar environment the results of this probe will also show, whether visual input per se can influence the quality of the spatial tuning of place cells when rat pups are exposed to a completely novel environment for the first time.

Table III-19: Overview of recorded cell numbers in the 'novel dark' probe across development. Given are numbers of complex spike cells (left column), place cells as defined for the analysis of single trial measures (middle column) and stability filtered place cells for the analysis of the across trial measures (right column).

	# complex spike cells	# place cells (according to 'spatiality')	# place cells (stability filtered)
P14-15	49	23	8
P16-17	72	46	17
P18-19	89	61	20
P20-21	70	37	14
P22-27	no cells	no cells	no cells
adult	105	87	64

Table III-19 gives an overview of the number of cells recorded for this probe trial type ('novel dark') for the respective age bins. Note that no data is available for post-weanling pups (P22-27) and that for the youngest age group (P14-15) only a rather limited amount of stability filtered place cells is available (n=8).

The response of place cells to an exposure to a novel environment in dark conditions is broadly very similar to the results of the novel light probe (see Figure III-38; compare to Figure III-35). At all ages the majority of place cells remap to some extent. Cells shift their firing fields to an unpredictable new location (cell 2 at P14-15, cell 2 and 3 at P16-17, cell 1 and 2 at P18-19, cell 2 and 3 at P20-21 and cell 2 at adults), they cease firing (cell 1 at adults), become active (no example shown) or even lose their spatial specificity (cell 3 at P18-19 and cell 1 at P20-21). There seems to be a general tendency of place cells to become somewhat fuzzier and show a larger area of firing at all ages (cell 1 at P14-15, cell 1 and 2 at P16-17, cell 3 at P18-19, cell 1 at P20-21 and cell 3 at adults). For all these trends there does not seem to be a developmental effect, but a rather comparable response for all age bins. Although there is no data for post-weanling pups for this probe trial, it is safe to assume that no different results would be expected for animals of this age.

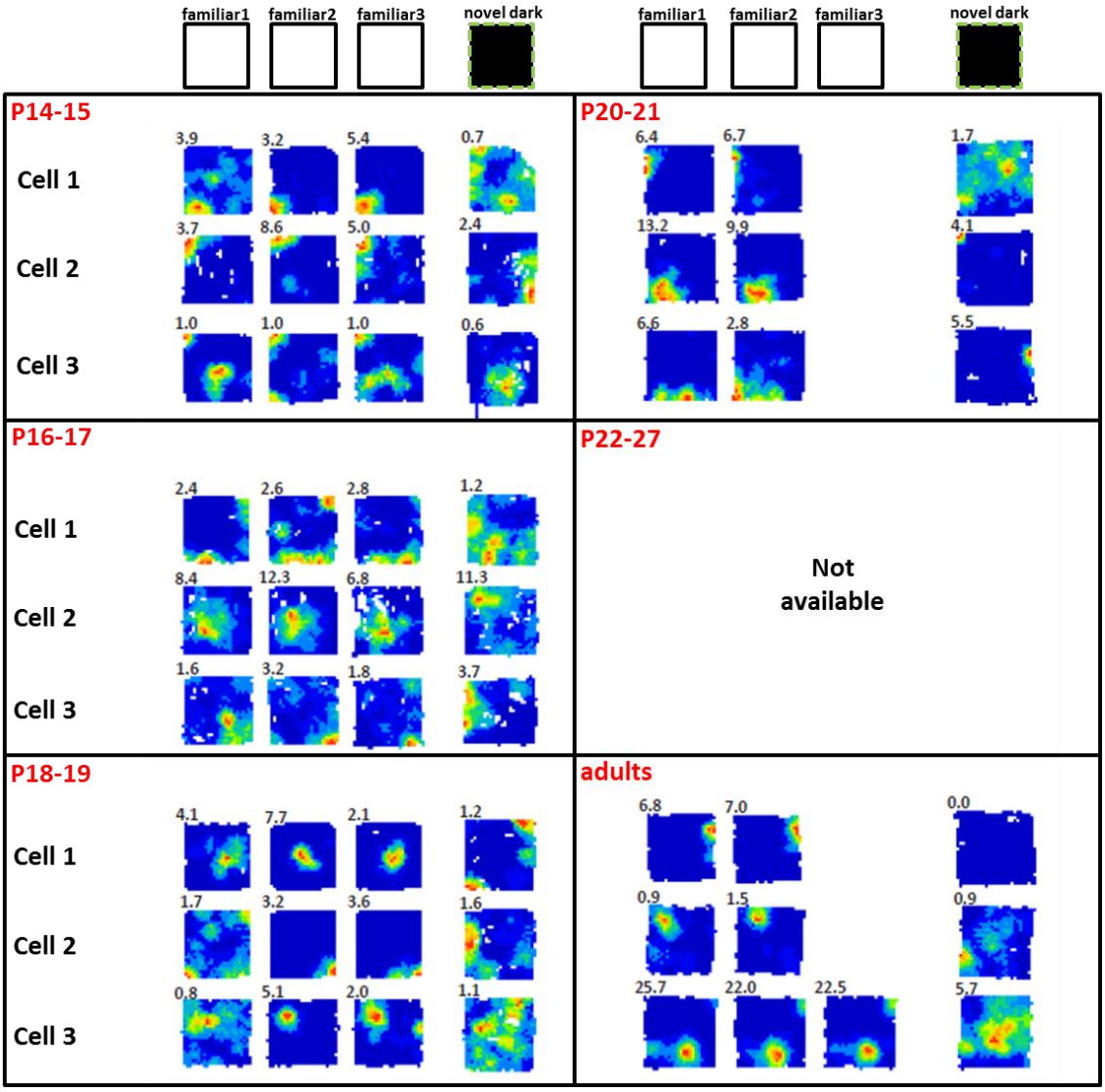


Figure III-38: Representative rate maps of place cells recorded inside the familiar environment and under 'novel dark' conditions across development (P14-15 – adults). For each age bin (black squares) 3 representative examples are shown across familiar trials ('familiar1-3') run before the probe trial and the probe trial itself ('novel dark'). Because this probe trial type usually constituted the last trial in a probe series no familiar trials after the probe are shown. All example cells shown have average spatial information scores across 'familiar1-3' in the range of age mean±standard deviation. Top row gives schematic of trial sequence. Rate maps are false colour auto-scaled heat maps with progressively warmer colours indicating progressively higher firing rates. Peak firing rates (Hz) are indicated at top left of each rate map. P(x)-(x) indicates age bin. Note that there is no data for P22-27.

Population analysis of place cell properties for the 'novel dark' probe

Quality of spatial firing during 'novel dark' probe

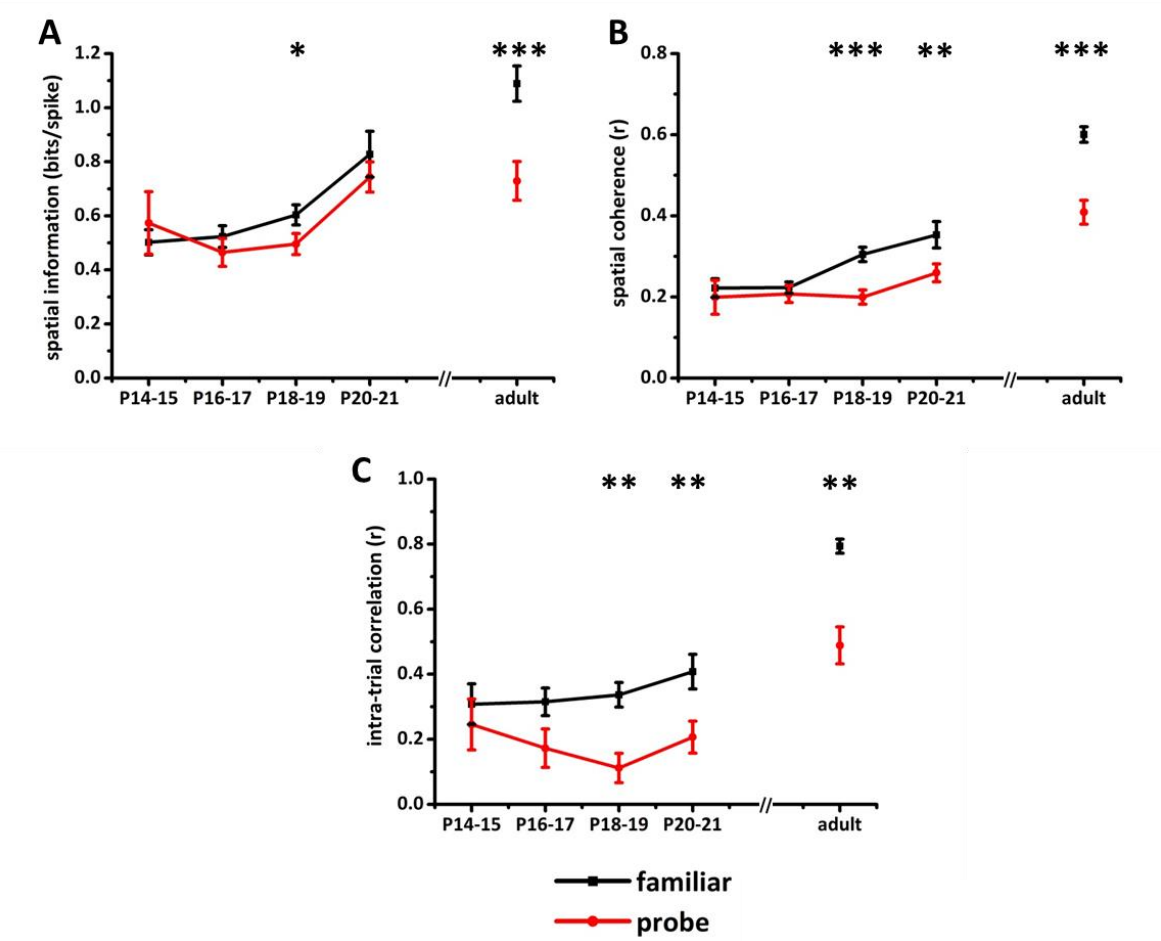


Figure III-39: Quality of the spatial signal of place cells recorded inside the familiar environment (black lines) and under 'novel dark' conditions (red lines) between P14-15 and adults. Depicted are spatial information (A, bits/spike), spatial coherence (B, r) and intra-trial correlation (C, r). Values for familiar environment are means across those trials inside the familiar environment before any probe trial was conducted. All values are population means±SEM. Note that there is no data for P22-27. *p<0.05, **p<0.01, ***p<0.001

The quality of the spatial firing is affected in an age-dependent manner during the 'novel dark' probe (see Figure III-39). All three parameters show a significant increase with age (spatial

information: $F_{4,157}$=7.98, p<0.001; spatial coherence: $F_{4,157}$=38.01, p<0.001; intra-trial correlation: $F_{4,157}$=36.01, p<0.001).

All three scores also reveal a main effect of environment (spatial information: $F_{1,157}$=9.97, p=0.002; spatial coherence: $F_{1,157}$=39.43, p<0.001; intra-trial correlation: $F_{1,157}$=30.68, p<0.001), as well as a significant interaction between age and environment (spatial information: $F_{4,157}$=2.52, p=0.044; spatial coherence: $F_{4,157}$=6.76, p<0.001; intra-trial correlation: $F_{4,157}$=2.87, p=0.025). From the pairwise comparisons it becomes clear that the interaction is caused by an absence of an effect during the probe trial at P14-15 and P16-17 on the one hand, and a significant decrease of all three scores for all older animals (see Table III-20). The only exception to this is spatial information at P20-21 which is non-significant.

This result is very interesting as it shows (as the 'novel light' probe, see Figure III-36) that novelty affects the quality of the spatial firing of place cell to different degrees between rat pups and adults. For young animals the spatial tuning remains completely unaffected during the probe trial.

Table III-20: Overview of results from pairwise comparisons for the interaction between age and environment for the quality of the spatial signal in the 'novel dark' probe across development. P-values are indicated for spatial information, spatial coherence and intra-trial correlation. Red values are below 0.05-significance level. Note that there is no data for P22-27.

	spatial information	spatial coherence	intra-trial correlation
P14-15	0.42	0.68	0.41
P16-17	0.39	0.57	0.29
P18-19	0.02	<0.001	0.001
P20-21	0.09	0.003	0.01
adults	<0.001	<0.001	0.001

Stability of spatial firing during 'novel dark' probe

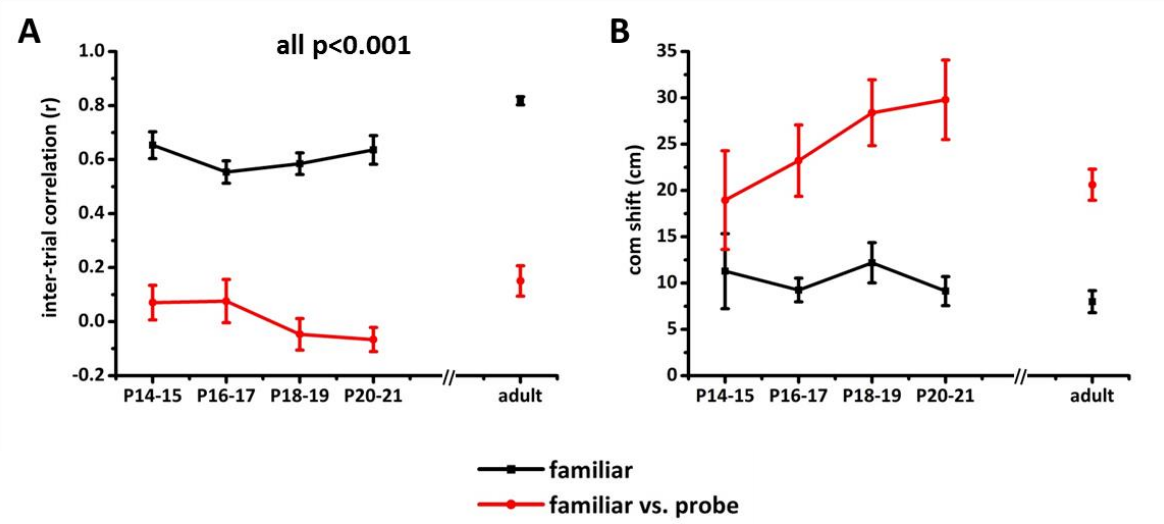


Figure III-40: Stability of the spatial signal of place cells recorded inside the familiar environment (black lines) and under 'novel dark' conditions (red lines) between P14-15 and adults. Depicted are inter-trial correlation (A, r) and centre of mass shift (B, cm). Note that no return-to-baseline correlation is available for this probe trial type because in most cases it was the last trial of a series. In A 'all p<0.001' indicates the results of the pairwise comparisons for the interaction between age and environment. All values are population means±SEM. Note that no data is available for P22-27.

The stability of place cell firing between familiar and probe trials shows a clear cut result (see Figure III-40). Inter-trial correlation shows a main effect of age while centre of mass shift does not show this effect (inter-trial correlation: $F_{4,79}$=9.85, p<0.001; centre of mass shift: $F_{4,79}$=1.43, p=0.23).

At all ages there is a massive reduction of inter-trial correlation and a massive increase in average centre of mass shifts during the probe trial (main effect of environment: inter-trial correlation: $F_{1,79}$=243.91, p<0.001; centre of mass shift: $F_{1,79}$=48.51, p<0.001). Inter-trial correlation furthermore also shows a significant interaction between age and environment ($F_{4,79}$=3.27, p=0.016), while the interaction is non-significant for centre of mass shifts

($F_{4,79}$=1.37, p=0.25). The pairwise comparisons for inter-trial correlation show significant differences for all age bins between the average familiar stability and the stability across recording environments (all p<0.001). Just as in the 'novel light' probe (see Figure III-35) the place cell representation undergoes profound changes when the firing fields of the familiar recordings are compared to the ones from the 'novel dark' probe. It is thus safe to conclude that regardless of age, rat pups, just as adults, can detect a completely novel environment even in darkness and their place cells form a novel representation.

Active vs. inactive cells during 'novel dark' probe

Table III-21: Overview of place cell numbers that are active in both environments and those that are either exclusively active during probe ('probe only') or familiar ('familiar only') trials for the 'novel dark' probe. Given are absolute numbers as well as proportion from the place cell population defined by their overall spatiality in a probe series in parentheses. Note that cells showing an inconsistent firing pattern across all familiar trials are excluded from this analysis.

	# both environments (%)	# probe only (%)	# familiar only (%)
P14-15	16 (70%)	0 (0%)	2 (9%)
P16-17	28 (61%)	3 (7%)	7 (15%)
P18-19	48 (79%)	5 (8%)	3 (5%)
P20-21	28 (76%)	8 (22%)	1 (3%)
P22-27	no cells	no cells	no cells
adult	44 (51%)	5 (6%)	29 (33%)

When looking at the numbers of place cells which are exclusively active in either familiar or probe trials a similar picture emerges as with recordings in a novel environment under light conditions (see Table III-21, compare to Table III-18). For all age groups there are cells showing exclusive activity in one of the two environments. Interestingly, there seems to be a trend for

adult animals to have a far higher fraction of cells ceasing activity during the probe trial than vice versa. For rat pups no such trend is observable. It is noteworthy that for the 'novel dark' probe there does not seem to be a developmental trend of showing higher numbers of place cells with exclusive activity in either environment with increasing age.

III.4.4.4 Summary of results from recordings in a novel environment

The results from a comparison of recordings inside a familiar and a novel environment clearly demonstrate that animals at every age bin are forming a novel spatial representation inside these novel environments, irrespective of whether visual cues are present or recordings are conducted in absolute darkness (see Figure III-37 and Figure III-40). More interesting are the effects on the quality of the spatial signal if compared between the familiar and novel environments. For both novel environment probes an effect is completely absent for data recorded from animals younger than P18 (see Figure III-36 Figure III-39). Interestingly, only recordings in a novel environment under dark conditions strongly affect place cell quality in animals aged between P18-P21 (see Figure III-39), while in light conditions an effect on place cell quality is mainly absent (see Figure III-36). For adult controls an effect on place cell quality is present in both probe trial types, although it seems that the reduction in quality is stronger for recordings inside the 'novel dark' environment (see Figure III-36 and Figure III-39). Unfortunately, hardly any data is available for post-weanling rat pups rendering it impossible to understand whether these animals show the consistent adult-like reduction in place cell quality. However, the few numbers of cells that are available for the 'novel light' probe seem to point out to similar effects as in adult controls in terms of a reduction in quality of place cell firing during the 'novel light' probe.

An interesting similarity can be found if the effect on place cell quality during probe trials is compared between 'novel dark' and 'familiar dark' (see Figure III-33 and Figure III-39). For rat pups the reduction in quality during the probes seems to emerge at a very similar time point in development (P18-P21). This supports the interpretation that the earlier emergence of a reduction in place cell quality for the 'novel dark' probe (in comparison to 'novel light') is

mainly caused by an absence of visual cues and not e.g. by chance due to the lower numbers of cells recorded under ‘novel light’ conditions (see Table III-17 and Table III-19).

III.5 'Knock-on' effects of probe trials

III.5.1 Introduction to 'knock-on' effects analysis

Some results from the above described probes need a more detailed analysis. It is somewhat puzzling that for pups the return-to-baseline correlations do not reach values similar to those of the average familiar correlation in the analysis of the 'new floor' and 'familiar dark' probe (see Figure III-28 and Figure III-34). In fact, the values for the respective return-to-baseline correlations are rather similar or even lower than the respective familiar vs. probe correlations. Although the data was pre-filtered for threshold stability inside the familiar environment (see Figure III-21 and Figure III-22) place cells in pre-weanling rat pups seem sometimes to become somewhat unstable after the probe trial was performed in comparison to the preceding trials inside the familiar environment. To analyse in more detail the underlying effects for this result, recorded place cells were characterised according to the relationship between average familiar correlation, return-to-baseline correlation and familiar vs. probe correlation. The threshold value for an unstable cell was set to be the 95^{th} percentile of inter-trial correlations based on spike-shuffled data (see Figure III-22). Cells were then classified accordingly (see Figure III-41 for an example of each cell type):

Type 1=all stable: return-to-baseline correlation and familiar vs. probe correlation > threshold

Type 2=probe only remapping: return-to-baseline correlation > threshold and familiar vs. probe correlation < threshold

Type 3=r2b only remapping: return-to-baseline correlation < threshold and familiar vs. probe correlation > threshold

Type 4=all remapping: return-to-baseline correlation and familiar vs. probe correlation < threshold

Type 5=not classifiable: other

In general type 1=all stable and type 2=probe only remapping represent cells which show stable familiar correlations and either remain stable during probe trials (type 1=all stable) or become unstable during probe trials (type 2=probe only remapping). These cell types do not show a 'knock-on' effect and will show a return-to-baseline correlation somewhat similar to the average familiar correlation. Type 3=r2b only remapping and type 4=all remapping represent cells which have an unstable return-to-baseline correlation and either are stable (type 3=r2b only remapping) or unstable (type 4=all remapping) between familiar and probe trial. These cell types do show a 'knock-on' effect (see Figure III-42 for examples of type 3=r2b only remapping and type 4=all remapping cells from each age bin).

The magnitude of this effect also seems to differ between probe trials (see Table III-22). But since the available data for each probe trial type differs quite a lot across probes and age bins, the results of all probe trials were pooled. Only those datasets were used where the respective probes were encompassed by two recording trials inside the familiar environment. The stability filtered place cell population for each probe was used for this analysis.

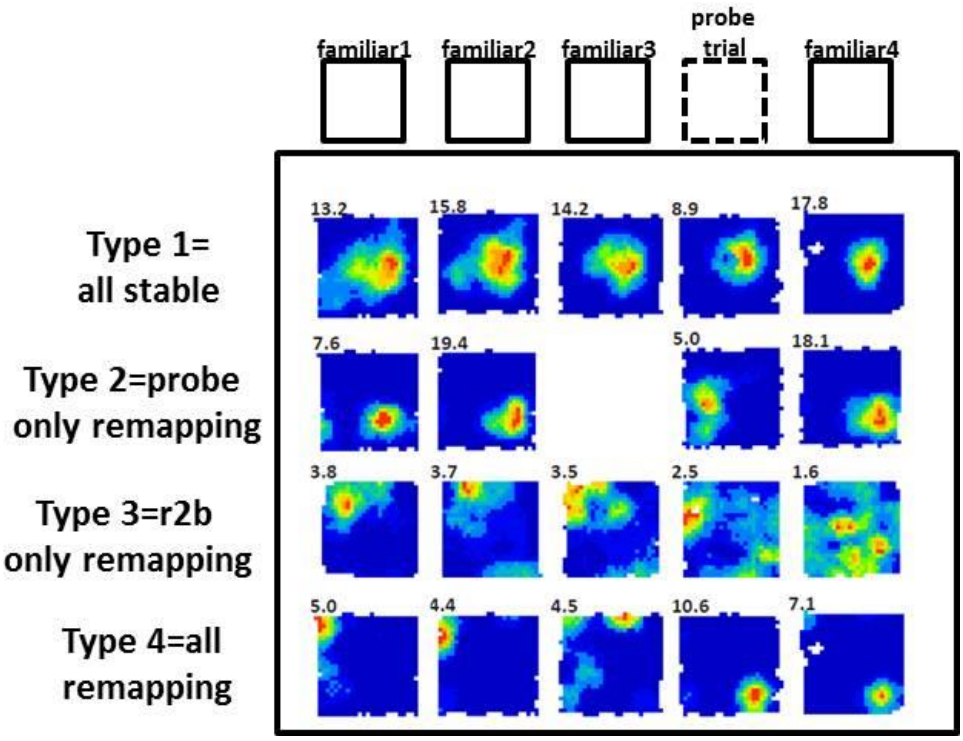


Figure III-41: Examples of different cell types in the 'knock-on' effects analysis. Shown is one example for each type (1-4). Top row indicates trial sequence. Note that probe trial can be a probe of any kind. Rate maps are false colour auto-scaled heat maps with progressively warmer colours indicating progressively higher firing rates. Peak firing rates (Hz) are indicated at top left corner of each rate map. All examples are actually recorded units in one of the probe series.

Table III-22: Overview of cell types (1-5) across different types of probes and development. For each age bin numbers for each cell type are indicated. 'Always last probe' indicates that generally recording data is available, but that the probe trial always constituted the last trial in an experimental series.

		new walls	new floor	new floor & new walls	familiar dark	novel dark	novel light
P14-15	type1	no data	2	no data	4	always last probe	no data
	type2	no data	1	no data	1	always last probe	no data
	type3	no data	1	no data	1	always last probe	no data
	type4	no data	1	no data	1	always last probe	no data
	type5	no data	8	no data	1	always last probe	no data
P16-17	type1	4	18	always last probe	11	always last probe	always last probe
	type2	2	2	always last probe	5	always last probe	always last probe
	type3	0	1	always last probe	6	always last probe	always last probe
	type4	2	2	always last probe	3	always last probe	always last probe
	type5	1	2	always last probe	3	always last probe	always last probe
P18-19	type1	2	13	1	12	always last probe	always last probe
	type2	0	4	2	2	always last probe	always last probe
	type3	0	1	0	7	always last probe	always last probe
	type4	1	3	1	2	always last probe	always last probe
	type5	0	1	1	2	always last probe	always last probe
P20-21	type1	13	3	1	18	0	always last probe
	type2	0	0	4	3	2	always last probe
	type3	1	0	0	0	0	always last probe
	type4	1	0	0	6	1	always last probe
	type5	0	0	1	5	0	always last probe
P22-27	type1	11	13	always last probe	5	no data	always last probe
	type2	0	3	always last probe	0	no data	always last probe
	type3	0	2	always last probe	0	no data	always last probe
	type4	2	1	always last probe	0	no data	always last probe
	type5	2	2	always last probe	1	no data	always last probe
adult	type1	33	77	43	57	6	always last probe
	type2	0	8	7	5	10	always last probe
	type3	1	0	0	0	0	always last probe
	type4	1	1	0	0	1	always last probe
	type5	2	11	9	11	16	always last probe

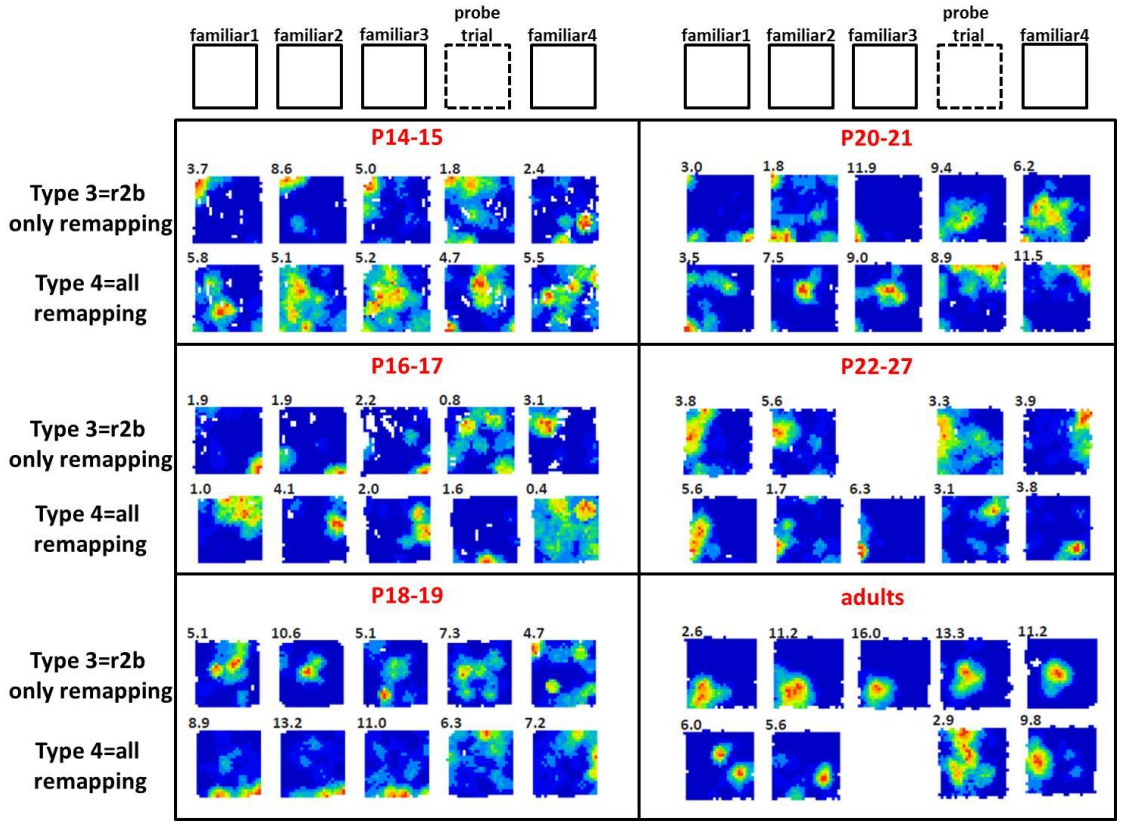


Figure III-42: Example rate maps of cells of type 3 and 4 across development ('knock-on' effects). Each age bin (black rectangles) shows one example of a type 3 and type 4 cell. Top row indicates general trial sequence. Note that probe trial can be a probe of any kind. Rate maps are false colour auto-scaled heat maps with progressively warmer colours indicating progressively higher firing rates. Peak firing rates (Hz) are indicated at top left corner of each rate map.

III.5.2 Results for 'knock-on' effects

When the proportions of each cell type (see Figure III-43B)) or the proportion of cells showing/not showing a 'knock-on' effect (see Figure III-43A)) are quantified, a clear developmental trend is observable. For pre-weanling rat pups (< P22) 20-30% of the place cell population shows some form of 'knock-on' effect after the probe trial. Interestingly the vast majority (ca. 70%) of those cells belongs to type 4=all remapping cells, meaning that they

remap during the probe trial compared to the preceding trial inside the familiar environment. Only a small fraction of cells (2-10% depending on age of the animals) shows a stable field during the probe trial, but then remaps during the subsequent familiar trial (type 3=r2b only remapping). Note that this fraction is larger for animals younger than P18 (P14-15: 17%, P16-17: 13%) and does not exceed 6% of the place cell population for any of the following age bins. For adult animals only a very small fraction (ca. 4%) of place cells shows a 'knock-on' effect. The amount of place cells showing the effect in post-weanling rat pups (ca. 10%) lies somewhat in between the proportions of pre-weanling pups and adult controls. This result clearly demonstrates that place cells in adult animals form a very stable representation of the familiar recording environment, which usually does not get perturbed by a probe trial. In contrast in rat pups a proportion of place cells tend to show some changes in the representation of the familiar environment after a probe trial was conducted. This type of analysis is essentially another way of showing the difference between return-to-baseline and average familiar correlations for some of the probe trials (see Figure III-25, Figure III-28 and Figure III-34).

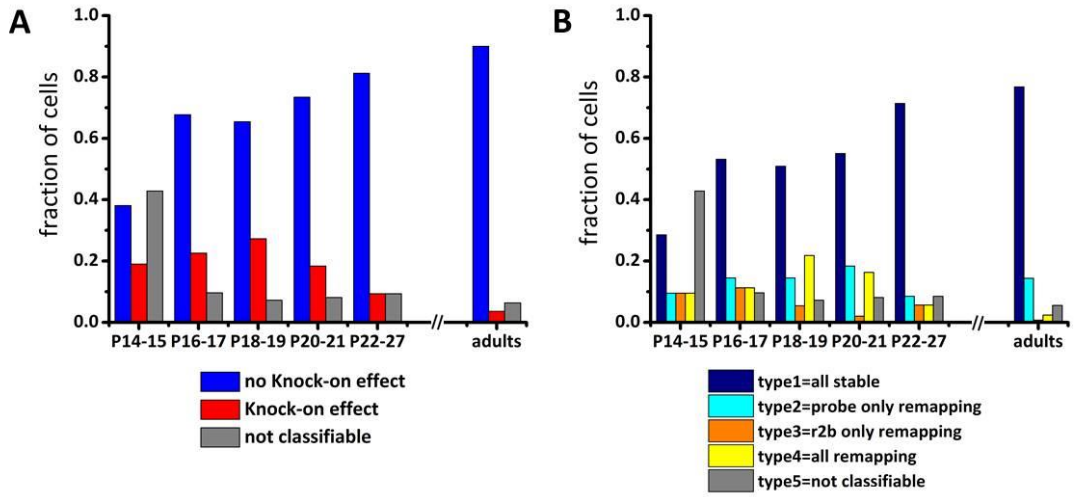


Figure III-43: Overview of fraction of place cells that show a 'knock-on' effect during probe trials across development (P14-15 – adults). A: Fraction of cells that either show no 'knock-on' effect (blue) or do show such an effect (red) or cannot be classified accordingly (grey). Note that to be classified as showing no 'knock-on' effect a cell had to be classified as type1 or type 2 on all probe trials in which it was recorded (the same logic applies to classifying a cell as showing a 'knock-on' effect only with type 3 and 4 cells). B: Fraction of cells belonging to individual cell types (type 1 – navy, type2 – cyan, type 3 – orange, type 4 – yellow and type 5 – grey). Note that the results from all probe trials were pooled for this analysis.

III.6 Histology

Locations of recording electrodes where confirmed with a standard Nissl-staining (see Figure III-44). In the figure two examples are shown for each respective recording location, except for the border of CA3-CA1. For rat pups place cells were recorded from CA3 (pups, CA3), CA1 (pups, CA1; pups, anterior CA1)) and in one animal the location of the electrodes was just at the border between CA3 and CA1 (pups, CA3/CA1). The recordings from CA1 in rat pups were conducted at anterior levels of the hippocampus (pups, anterior CA1) as well as more posterior locations (pups, CA1). In adult animals recording electrodes were always aimed at more posterior parts of the hippocampus (adult, CA1).

Table III-23 shows a detailed overview of the number of animals per recording location and the respective place cell yield.

Table III-23: Overview of recording locations across animals and respective place cell yield. Note that number of place cells refers to place cells recorded in familiar environment before any probe trial was conducted.

	# animals	recording location	# place cells
rat pups	2	CA3	65
	1	CA3/CA1	28
	2	anterior CA1	71
	22	posterior CA1	239
adult controls	10	posterior CA1	116

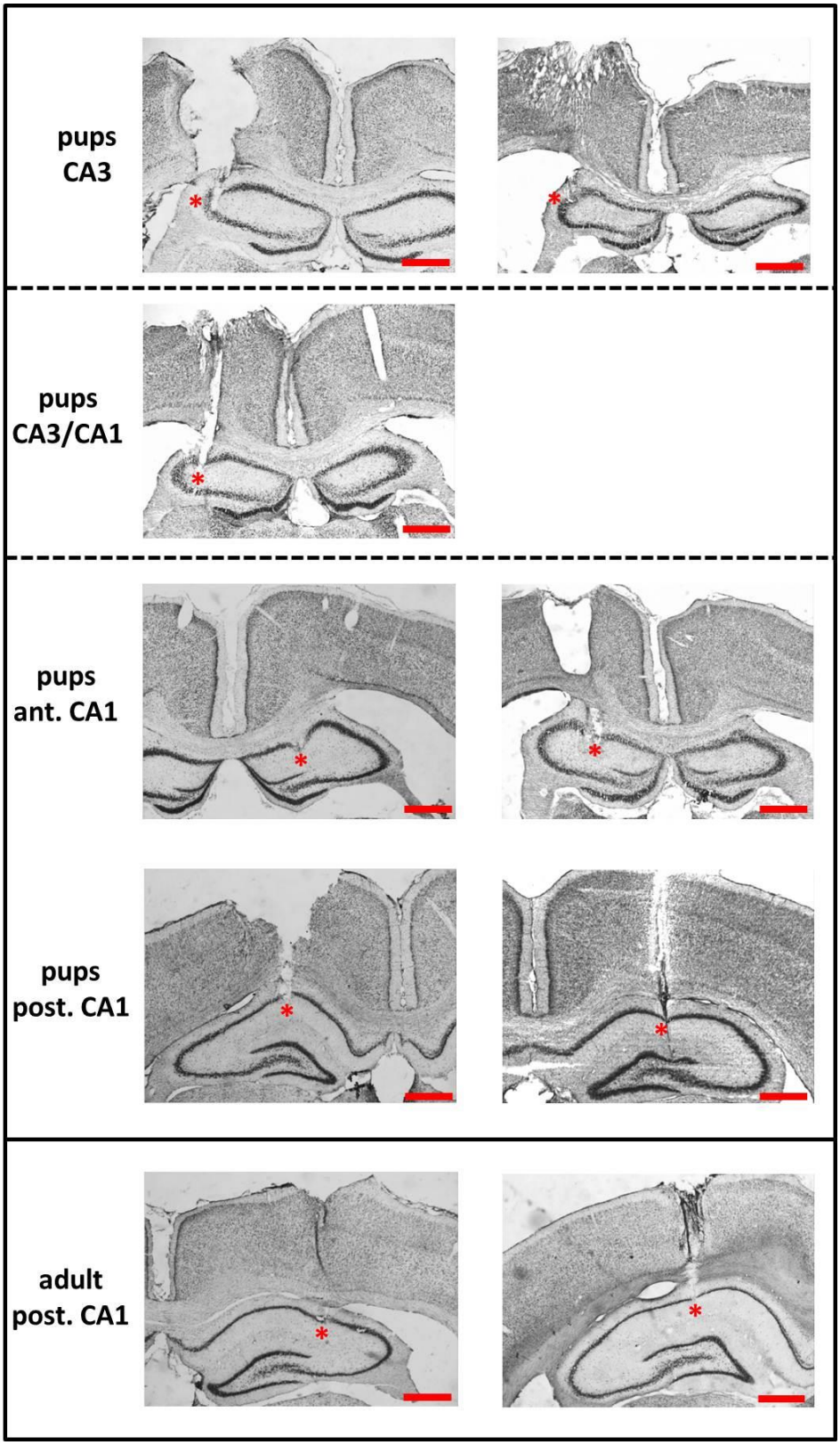


Figure III-44: Examples of recording positions. For each respective recording location two examples are shown (except for CA3/CA1). Red asterisks mark locations where electrodes penetrated cell layer. Red bar indicates 1 mm. ant.: anterior

IV Discussion

IV.1 Summary of results

This first section of the discussion will give a summary over the results found in this work. First the key findings of this thesis are briefly presented followed by a detailed summary of all results. The latter part is split into a summary of the functional maturation of place cell properties inside the familiar environment and the responses of place cells during the sensory manipulation trials.

IV.1.1 Key findings

The results of the work presented in this thesis can be broadly summarised into three key findings:

A replication of results previously published by others (Langston et al., 2010; Wills et al., 2010) concerning the functional maturation of place cell properties in a familiar environment. The functional maturation of place cell properties regarding their 'spatiality' (spatial information, spatial coherence and intra-trial correlation) or stability (inter-trial correlation and centre of mass shift) across age measured inside the familiar environment found in this work shows a very good match with those published recently (Langston et al., 2010; Wills et al., 2010).

An extension of those previous findings by showing that place cells in young rat pups react overall quite similar to those of adults after a sensory manipulation of the familiar environment. There is e.g. an influence of the presence of visual information even in the youngest animals despite the immaturity of that sensory system at these ages. However, there seems to be a slightly stronger effect of the manipulation of local tactile/olfactory intra-maze

cues on place cell firing in early development compared to post-weanling and adult animals (compare 'new floor & new walls' probe with 'familiar dark' probe). Moreover, some evidence for configural properties of place cells recorded from young rat pups was presented as well (lack of strong effect on place cell stability during 'new walls' and 'new floor' probe), showing that place cells seem to be inherently tuned to integrate information from multiple sensory cues, and therefore work as a higher-order representation of space even at the youngest ages.

The finding that place cells in pre-weanling rats, but not post-weanling or adult animals, show an increased stability across recording trials when located closer to an environmental boundary. There was good evidence presented in this thesis that this constitutes a true property of place cells in development (until ca. age of weaning – P21). This highlights the importance of environmental boundaries as stabilising factors for place cell firing and also has some theoretical implications. As place cell firing can be modelled using the input of several boundary sensitive cells (BVCs) (Barry et al., 2006; Hartley et al., 2000), this raises the question whether early in development, before the emergence of functional grid cell firing in MEC, these BVCs might form the main functional input to place cells.

IV.1.2 Place cell properties in a familiar environment

All properties of place cells analysed in this thesis (spatial information, spatial coherence, intra-trial correlation, mean and peak firing rates, field size, number of subfields, inter-trial correlation and centre of mass shift) showed a developmental functional maturation between pre-weanling rat pups and adult animals (see Figure III-14, Figure III-15, Figure III-16, Figure III-17 and Table III-5). Nearly all of these properties develop in a more or less linear fashion across the age bins used in this study (P14-15, P16-17, P18-19, P20-21, P22-27 and adult controls). The only property that showed a non-linear trend of change across development was the

reduction of the number of firing fields per cell (see Figure III-16B). Here, a rather sharp and sudden drop occurred at some point between P22-27, most likely around P24. It is noteworthy that the above-mentioned place cell properties seem to mature to a different extent until P27. Field size, mean firing rate, number of firing fields and centre of mass shift all show adult-like levels between P22-27, while the quality of the spatial tuning must undergo some further maturation processes after P27 (see Figure III-14). The average stability of place cells is also still significantly different from adult levels at P22-27. These results highlight the change in the location specific firing of place cells in developing rats over the third and fourth week of the animals' life. In young rats (<P18-19) place fields tend to be more 'fuzzy', larger, contain more firing fields and have a much reduced stability across recording trials when compared to post-weanling rat pups (P22-27) and adult animals (see Figure III-13).

The most interesting and most striking new finding in this work for the baseline properties of place cells consisted of describing an effect of environmental boundaries on place cell stability in pre-weanling, but not post-weanling rat pups or adult animals (see Figure III-18). In pre-weanling animals, fields located closer to an environmental boundary tend to be more stable than those further away. In post-weanling rat pups and adult animals no such dependence of place cell stability on field-to-wall distance could be observed. It could be shown conclusively that this seems to be a genuine developmental effect (see Figure III-19, Figure III-20 and Table III-6), and it is intriguing that the time point when the dependence of the two parameters disappears, coincides with the emergence of grid cell firing in MEC (Wills et al., 2010).

IV.1.3 Place cell properties during sensory manipulation trials

To understand whether sensory integration on place cells differs between developing rat pups and adult animals several types of sensory manipulation trials were conducted. In these, parts ('new walls', 'new floor', 'new floor & new walls' and 'familiar dark') or all ('novel dark' and 'novel light') of the local intra-maze cues (and distal cues for novel environments) were removed/changed from/to the familiar environment, thus probing the responses of place cells in the face of varying degrees of dissimilarity to a highly familiar context (see Figure III-23, Figure III-26, Figure III-29, Figure III-32, Figure III-35, Figure III-38).

IV.1.3.1 Stability of place cells

As far as the stability of place cells during these probe trials is concerned, the responses of place cells at every age bin were overall rather similar to those of adult animals with some small differences (Figure III-25, Figure III-28, Figure III-31, Figure III-34, Figure III-37 and Figure III-40). When the walls of the familiar environment were replaced for a visually identical copy, place cell representations did not change significantly compared to the familiar context at any of the age bins, although a trend for a reduction in place cell stability was present especially for very young animals (see Figure III-25). In contrast, replacing the floor induced remapping in parts of the place cell representation (see Figure III-28). Surprisingly, this effect was mainly seen in post-weanling rat pups and adult control animals, while for pre-weanling rat pups this effect was only present at P18-19 and absent for all other age bins containing data from pre-weanling rat pups. Replacing both walls and floor in one single probe trial lead to strong remapping of the place cell representation at all age bins (see Figure III-31). These results

indicate that place cells in pre-weanling animals need to be confronted with more drastic changes of the local intra-maze cues compared to adult animals to induce changes in the spatial representation of an environment. This seems to indicate developmental differences in the detection of novelty.

Removing visual cues while animals explored the familiar environment lead to a moderate change in the spatial representation of the familiar context, which also seems to be independent of the age of the animals (see Figure III-34). Interestingly, this indicates an early dependence of vision on place cell stability, at a time where the eyes of the animals have just opened (Altman and Sudarshan, 1975; Bolles and Wood, 1964; Fagiolini et al., 1994; Foreman and Altaha, 1991; Moye and Rudy, 1985; Prévost et al., 2010; Routtenberg et al., 1978).

Together all of these results indicate that i) place cells are inherently configural and only require parts of the sensory input of the original familiar context and ii) that their stability seems to depend to comparable degrees on the presence of certain sensory cues in the environment across development.

Exposing animals to a completely novel environment generally lead to a more or less complete remapping of the place cell representation, irrespective of the age of the animals and irrespective of whether visual cues were present or not (see Figure III-37 and Figure III-40). This shows that even animals as young as P14-15 can detect drastic changes in the environment and form a novel spatial representation under such conditions.

IV.1.3.2 Spatiality of place cells

In comparison to the stability of place cells during sensory manipulation trials, the quality of the spatial tuning was much more affected in an age-dependent manner compared across all

probe trials (see Figure III-24, Figure III-27, Figure III-30, Figure III-33, Figure III-36 and Figure III-39). While the spatial tuning was unaffected during the 'new walls' probe at any age bin (see Figure III-24), reductions in the quality of the location specific firing of place cells were present in at least one age group in all other probe trials (see Figure III-27, Figure III-30, Figure III-33, Figure III-36 and Figure III-39). When the floor of the familiar environment was replaced for a visual replica, only place cells recorded from adult animals were strongly affected (see Figure III-27). Replacing both walls and floor resulted in a rather broad reduction in the quality of place cell firing across all ages, except for the spatial information content, which was only affected in adult animals (see Figure III-30).

Removal of visual cues had the most interesting effects on place cell quality. For all properties included in this analysis there was a clear age-dependent reduction (see Figure III-33). While in animals younger than P18 this had no impact, the influence of an absence of visual input increased until P22-27, where adult-like effects could be observed. This seems to support the interpretation that influence of visual input increases in parallel with the maturation of this sensory system and emerges very early in development (Akers et al., 2011; Fagiolini et al., 1994; Moye and Rudy, 1985; Prévost et al., 2010; Rudy et al., 1987). The recordings in a novel environment under dark conditions further corroborate these findings as here the age-dependency of an effect on place cell quality was very similar (see Figure III-39).

Place field quality in a novel environment, under light conditions, was strongly affected in adult animals, but not pre-weanling rat pups (see Figure III-36). Unfortunately, no data is available for post-weanling rat pups for this probe trial. This indicates that young animals do not yet show the experience-dependent improvement of their spatiality in a novel environment (Brun et al., 2008a; Cacucci et al., 2007; Frank et al., 2004; Wilson and McNaughton, 1993).

IV.1.4 Knock-on effects

Place cells in pre-weanling rat pups occasionally showed an influence of a probe trial on the subsequent familiar trial (see Figure III-26, Figure III-28, Figure III-32, Figure III-34, Figure III-43 and Table III-22). That is, they either showed some form of remapping during the probe and maintained this change in the following familiar trial, or place cells remapped in the familiar trial after the probe, although they showed a more or less stable field during the probe. It is unclear what exactly the underlying mechanisms are, but the effect seemed to depend on the type of probe trial. Sufficient data is only available for the 'new walls', the 'new floor' and the 'familiar dark' probe. Only the latter two types of probe trials induced this effect in some place cells (see Figure III-28, Figure III-34 and Table III-22). The effect was mainly present in pre-weanling rat pups and almost completely absent in adult animals (see Figure III-43). This indicates that a spatial representation of a highly familiar environment is much more stable over time in adult animals, which is supported by the fact that in young rat pups some forms of remapping also occasionally occur across familiar trials before any probe trial was conducted (see Figure III-23, Figure III-26, Figure III-35 and Figure III-38 for some examples).

IV.2 Pre-processing of the data

IV.2.1 Clustering of complex spike cells and interneurons

To separate complex spike cells from interneurons a similar approach to Csicsvari et al. (1999) was taken. Three parameters of spiking properties (spike width, first moment of 20ms-autocorrelation and mean firing rate) were used with a k-means based clustering algorithm (see Figure III-1 and Figure III-2). These parameters should differ substantially between both cell types, as both groups are markedly different in their action potential waveforms, burst properties and average firing rates (Fox and Ranck, 1975; Ranck, 1973). Especially for data recorded from adult animals this method seems to work well (see Figure III-2B). For data obtained from rat pups the separation of the two cell types seems to be less clear cut (see Figure III-2C). A reason for this might be the fact that complex spike cell waveforms are slightly narrower in young animals, while those of interneurons are wider (compared to adult controls) (see Figure III-3 and Table III-2). Furthermore, the differences in average firing rates between complex spike cells and interneurons are larger in adult animals. Hence, for rat pups the average distances in cluster space between both cell types might overall be shorter, which could lead to a slightly worse separation compared to adult animals. However, this should not have a great impact on subsequent data analyses, because in a second step only complex spike cells with a significant spatial tuning were selected and deemed place cells (see Figure III-4, Figure III-12 and Figure III-21).

IV.2.2 Ratio of complex spike cells to interneurons

It is not clear why the proportion of complex spike cells from the cell population in CA1 is slightly higher on average in rat pups compared to adults (see Table III-1). The proportions of complex spikes in the CA1 layer of the hippocampus found in adult animals (ca. 78%) is in line with previous studies, which found ca. 82% (Csicsvari et al., 1999) and ca. 80-90% (Wills et al., 2010). In another study, Aika and colleagues found that ca. 7% of the cell population in CA1 consisted of GABAergic neurons (Aika et al., 1994). Thus, the proportions found for rat pups might simply lie in the normal physiological range. It is noteworthy that the principal cell layer is generally thicker in rat pups (Frotscher and Seress, 2007) (see Figure III-44) and that interneuron migration in the hippocampus is not fully complete until at least P15 (Danglot et al., 2006). Thus it cannot be excluded that the different proportions actually represent a genuine developmental effect.

IV.2.3 Developmental change in spiking properties

While complex spike cells showed a more gradual change of their spiking properties, those of interneurons did not change between pre- and post-weanling rat pups, but differed to the ones from adult controls (see Figure III-3 and Table III-3).

These changes might reflect maturational processes on the level of ion channels and single cell physiology in postnatal development (Battistin and Cherubini, 1994; Bekenstein and Lothman, 1991; Durand et al., 1996; Harris and Teyler, 1984, 1983; Insel et al., 1990), but extra-cellular recordings are not the right approach to investigate such effects. Also the average values for

these properties obviously depend on the quality of the preceding k-means based clustering according to cell type. As this seems to be less accurate in rat pups (see section III.2.1.2), one has to be cautious with the interpretation of the possible underlying effects for the developmental change in spiking properties of complex spike cells and interneurons.

However, some conclusions can probably be drawn from the developmental change in these properties in both types of hippocampal cells. First, complex spike cells seem to show a stronger tendency to fire bursts of action potentials across development, since their first moment decreases from pre- to post-weanling rat pups, which is when adult-like values are reached (see Figure III-3, Table III-2 and Table III-3). Second, the amount of low rate firing interneurons (Fuentealba et al., 2008) must decrease dramatically some time after the end of the fourth week, as the average firing rate of interneurons roughly doubles between post-weanling pups and adult animals (see Figure III-3 and Table III-2).

IV.2.4 Proportions of place cells from complex spike cell population

The proportions of place cells from the complex spike cell population are similar to those found until P24 in Langston et al. (2010), while in Wills et al. (2010) proportions found after P18 are generally higher. Both studies also report an increase in place cell proportions across development (much more convincingly shown in Wills et al. (2010)). In contrast, in this thesis more or less stable proportions for all age bins containing rat pup data were found (see Figure III-5 and Table III-4). Note that Wills et al. (2010) used a more strict criterion of generally defining complex spike cells (visual inspection of temporal autocorrelogram and minimum spike width). Since complex spike cells in younger animals fire at lower average rates, this will

make the autocorrelation functions much more prone to noise. This might lead to the exclusion of these units in the study by Wills et al. (2010). On the other hand, Langston et al. (2010) used a more similar approach to this thesis of defining complex spike cells and the proportions are also more similar. Scott et al. (2011) also report an increasing probability between P22-43 of hippocampal pyramidal cells being place cells, but since their method of place cell definition (threshold for spatial coherence) was substantially different to the one employed in the other studies and this thesis, it is difficult to directly compare results.

IV.3 CA1 vs. CA3

Since a number of place cells were recorded from CA3 (n=67 across age bins P14-15, P16-17, P18-19, P20-21) and CA1 (n=276 across age bins P14-15, P16-17, P18-19, P20-21), the first step of the data analysis of place cell properties was to compare their properties. From adult animals it is known that place cells in CA1 and CA3 do not differ substantially in their basic properties (Barnes et al., 1990; Leutgeb et al., 2004; Muller et al., 1987; Renaudineau et al., 2007), but show differences in their population dynamics (Lee et al., 2004; Leutgeb et al., 2005b, 2004; Vazdarjanova and Guzowski, 2004).

In this study no marked differences in various place cell properties (spatial information, intra-trial correlation, mean and peak firing rates and field size, inter-trial correlation, centre of mass shift) between place cells recorded from CA3 or CA1 in the familiar environment could be found (see Figure III-8, Figure III-9, Figure III-10 and Figure III-11). The only significant differences were found for spatial coherence at P18-19 and P20-21 (see Figure III-8B) as well as for the number of subfields at P20-21 (see Figure III-10B). Despite this, it seems reasonable that just as for adult animals, immature rat place cells in CA1 and CA3 do not differ substantially in their basic properties in a familiar environment. This is because none of these statistical differences point out to systematically different functional properties of place cells recorded from the two CA subfields. Furthermore place cells recorded from CA3 and CA1 did not differ in their average spatial information content (see Figure III-8A) nor their average inter-trial stability (see Figure III-11A), which both belong to the most widely used parameters to describe quality and stability of the location specific firing of place cells. That is why data recorded from both CA subfields were pooled for the analysis in this thesis, a practice often

employed for the analysis of adult data (Bostock et al., 1991; Renaudineau et al., 2007; Save et al., 2000; Siegel et al., 2008; Tanila et al., 1997b; Wilson et al., 2004).

These findings nonetheless do not rule out any difference in the functional maturation of CA1 and CA3. Recordings in only a familiar environment are not able to investigate a possible difference, given from what is known from adult animals (Leutgeb et al., 2004; Vazdarjanova and Guzowski, 2004). Finally, to properly address the question whether place cells in CA1 and CA3 differ in their functional development, parallel recordings of units from both subfields in the same animal would be necessary. However, as investigating this was not the aim of this thesis, pooling of the data across CA1 and CA3 for the baseline analysis will not have confounded the results presented in this study.

IV.4 Place cells in a familiar environment

IV.4.1 Development of place cell properties corroborates previous findings

The population analysis of the developmental change in various place cell properties in this study in general confirms results from previous work (Langston et al., 2010; Scott et al., 2011; Wills et al., 2010). All place cell properties presented in this work showed a significant change throughout development (see Figure III-14, Figure III-15, Figure III-16, Figure III-17 and Table III-5). When the respective values obtained in this study for spatial information, spatial coherence, intra-trial correlation and inter-trial correlation are compared to corresponding ages in previous work, the results overall show a good match (compare Figure III-14 and Figure III-17A with Figure 3 in Wills et al. (2010) and Figure 2 in Langston et al. (2010)). Particularly the ones from Wills et al. (2010) are nearly identical with what was found in this work. Note that in Langston et al. (2010) data is binned across three days and not across two as in Wills et al. (2010) and in this thesis (at least for animals younger than P22), which makes it difficult to directly compare place cell properties at each individual age bin. This will furthermore also affect the threshold values of spatial information for individual age bins used to classify a unit as place cell, and might explain the difference in spatial information content in animals younger than P20 in the Langston et al. (2010) paper.

The different binning cannot however explain the difference in spatial coherence between this work and Wills et al. (2010) on the one hand, and Langston et al. (2010) on the other. The latter study presents unusually high values for spatial coherence even for adult data (Park et al., 2011; Save et al., 2000) and from their methods section, it is not entirely clear whether this

study used the exact same approach for calculation of spatial coherence as this work and Wills et al. (2010).

In this study an increase in mean as well as peak firing rates across development was found (see Figure III-15). While for pre-weanling pups (< P21) only a trend, albeit not statistically significant, for increasing firing rates is present, there is a strong (especially for peak firing rate) increase between P18-19 and P22-27 (see Figure III-15 and Table III-5). Both Wills et al. (2010) and Langston et al. (2010) do not find such an increase, although in the former study adult controls have mean firing rates more than twice as large as animals at P28. Wills et al. (2010) however report a general increase in mean firing rates across all CA1 units, but not for place cells (see Figure S14), while Langston et al. (2010) only report a non-significant correlation between mean firing rate and age in CA1 (see supplementary information) without presenting any actual firing rates. Both studies do not report any peak firing rates. The exact reason for this difference is unclear, but it is known that firing rates of place cells (in adults) are generally highly variable (Lever et al., 2002). Moreover both studies also differ in their results on changes in firing rates of grid cells in MEC across development. It is noteworthy that Scott et al. (2011) also reports an increase in in-field and peak firing rate for place cells between P23-45.

Additional to what previous studies reported, this work also showed that the size of the main firing fields decreases across development (see Figure III-16A), which is in contrast to what was published previously by Scott et al. (2011). However, it is unclear how in Scott et al. (2011) both field size and spatial information can increase in parallel as these parameters are highly correlated (larger fields have lower spatial information contents).

A final general remark concerns the rather protracted development of place cell function described in the study by Scott et al. (2011). In this study examples of adult-like place cell firing could only be observed at P23, and furthermore develop to adult-like levels only around P40-45. No place cells were found prior to P23. What makes it difficult to directly compare results,

is the different approach in the analysis in the study by Scott et al. (2011). These authors do not present population means for individual age bins, but show the functional development for individual animals. Their results are nonetheless in stark contrast to those of this thesis and those of other studies which found emergence of place cell firing at P14-15 (this thesis) and P16 respectively (Langston et al., 2010; Wills et al., 2010). Moreover, in the latter studies and in this thesis some place cells with adult-like properties were already present in the youngest animals (see e.g. cell 2 at P14-15 in Figure III-13 or cells 1 and 3 at P14-15 in Figure III-32). It is also noteworthy that place cells recorded in this thesis recorded at P22-27 are already very similar to those of adult animals (see e.g. Figure III-13). The exact reason for these discrepancies remains unclear, but an emergence of place cell firing at P23 can confidently be excluded from the results of this thesis.

IV.4.2 Following the same place cells over multiple days

The above discussed functional maturation of place cells is essentially only a description of the change of averages for properties of place cells recorded across different animals. Ideally, one would want to try and observe the very same unit(s) across multiple days in the same animal. Unfortunately, this is technically very challenging, since due to the growth of the animals, a steady passive displacement of the recording electrodes occurs. This renders following the same place cells across several days almost impossible by causing a change of the unit positions in the cluster space (for spike sorting). To properly address this question, an imaging approach should be taken to follow the functional development of large populations of place cells in the same animal, as it has recently been shown for place cells in adult mice (Ziv et al., 2013).

IV.4.3 P14-15 demarcates emergence of place cell firing

This study is to the author's knowledge the first to show place cell recordings in animals younger than P16. At P14-15 place cells do already show some form of location specific firing (average spatial information content: 0.53±0.03 bits/spike), but their average stability across recording trials is rather poor (ca. 0.33±0.05) (see Figure III-14A and Figure III-17A). It is only slightly larger than the 95th percentile of the null distribution based on spike shuffled data for this age bin (ca. 0.28) (see Figure III-22). It thus seems that, although there are examples of adult-like place cells at this age (see e.g. cell 2 at P14-15 in Figure III-13), the hippocampus' ability to form a stable spatial representation of an environment is rather limited at this point in development. This is in line with the behavioural development of rat pups, as P14-15 denotes a time point before pre-weanling pups first start to leave the litter huddle (Gerrish and Alberts, 1996; Loewen et al., 2005) and start to show long consistent patterns of quadrupedal walking (Altman and Sudarshan, 1975; Bolles and Wood, 1964; Westerga and Gramsbergen, 1990). At this point in development there would be no need for the hippocampal system to form a stable map-like representation of the animals' environment, as young rats could home back to their nest from brief excursions by using only olfactory cues (Altman and Sudarshan, 1975; Cornwell-Jones and Sobrian, 1977).

Unfortunately P14-15 also probably represents the youngest age at which standard place cell recordings are methodologically possible, as even younger animals show very immature locomotion (Altman and Sudarshan, 1975; Bolles and Wood, 1964; Westerga and Gramsbergen, 1990) and hardly ever leave the litter huddle (Bolles and Wood, 1964; Gerrish and Alberts, 1996; Loewen et al., 2005). This means at this age, rats do not yet show their typical exploratory behaviour, and thus their poor coverage of a standard size laboratory environment would render it impossible to properly assess location specific firing of place cells.

One possibility might be to try and record place cells at ages younger than P14 in an 'aversive' environment, as there is one report of adult-like locomotion on a refrigerated surface at P4 (Altman and Sudarshan, 1975). It would certainly be interesting to investigate whether locational specific firing of place cells occurs abruptly at some point in development around P14 or whether under certain conditions it could be elicited in even younger animals.

IV.4.4 Place cells are stabilised by boundaries in pre-weanling rat pups

One of the most striking findings of this thesis was the dependence of place cell stability on field-to-wall distance in pre-weanling rat pups. This effect is absent in post-weanling rats and in adult animals (see Figure III-18). From recordings in adult animals it is well known that boundaries play an important role in place cell firing (Barry et al., 2006; Hartley et al., 2000; Lever et al., 2002; O'Keefe and Burgess, 1996). The relationship of distances between individual boundaries determines field shapes and field positions in an environment (O'Keefe and Burgess, 1996) and their removal can lead to remapping (Lever et al., 2002) or disintegration of place fields (Barry et al., 2006). In this thesis a general higher spatial tuning of place cells that are located close to a boundary was found, but this effect was present for all ages (see Table III-6). In contrast, only place cells recorded from pre-weanling rat pups showed increased stability, when their firing fields were located close to an environmental boundary. The abrupt disappearance of the effect at a time point, when grid cell firing emerges in the entorhinal cortex (Wills et al., 2012, 2010), seems to indicate that in pre-weanling rat pups other spatially modulated cells like e.g. BVCs in the subiculum (Barry et al., 2006; Hartley et al., 2000; Lever et al., 2009) or border cells in MEC (Solstad et al., 2008) could provide stabilising

input onto place cells. The BVC-model originally proposed that BVCs could be tuned to different distances from environmental boundaries (Hartley et al., 2000), but the majority in fact fire close to boundaries in adult animals (Lever et al., 2009). This also seems to be true for border cells in MEC (Solstad et al., 2008). This tuning of BVCs and border cells might be even tighter in rat pups in terms of distance to boundaries, due to e.g. shorter vibrissae in young animals (Landers et al., 2006), smaller body size or poorer visual acuity (Fagiolini et al., 1994).

Is it possible that pre-weanling rat pups simply use local cues on the environment walls to stabilise place fields? Although it cannot be ultimately excluded, it seems unlikely that these cues play an instrumental role for place cell stability in pre-weanling rat pups. When the walls of the familiar environment were replaced, little effect on place cell stability was found for most age bins with a possible exception at P16-17 (see Figure III-25). Animals might still use those cues, but if they played an important role a stronger effect on place cell stability would be expected in this probe trial.

A critical test for the validity of the hypothesis that BVCs or border cells provide stabilising input to place cells in CA1 in early development would be to try and record these cell types in very young animals and determine the time point of their emergence as well as describing their functional maturation. To corroborate the hypothesis two important points would have to be demonstrated by such experiments. First, these cell types need to appear earlier or at least at the same time as place cells in CA1 and show stability of their firing as soon as they emerge. Second, a manipulation of the integrity of BVC firing (e.g. lesion or temporary inactivation) should result in a destabilisation of place cells whose firing fields are located close to a boundary, thus establishing a functional link between these two cell types. Furthermore anatomical studies in young rats could provide further support about functional connections from subiculum to CA1 via entorhinal cortex or via a direct back-projection in early

development, which has been shown (via entorhinal cortex) for adult rats (Kloosterman et al., 2004).

IV.4.5 Does the emergence of grid cells (P21-24) demarcate a special time point for place cell firing?

Previous experiments showed that functional grid cell firing emerges around P21 in rat pups and develops rapidly afterwards reaching adult-like properties around P21-24 (Wills et al., 2012, 2010). MEC constitutes one major input onto place cells in CA1 (Steward and Scoville, 1976; Witter, 1993) and input from grid cells is furthermore believed to be (at least to some degree) an important factor for the formation of the unitary firing fields of place cells in the hippocampus (Blair et al., 2008; McNaughton et al., 2006; Solstad et al., 2006). It has recently been shown that all types of cells in MEC, non-spatial as well as grid cells, HD cells, border cells, and spatially periodic cells (Krupic et al., 2012) project to CA1 (Zhang et al., 2013). Interestingly, the majority of the input originated from non-spatial cells in MEC. The exact targets in CA1 of these projections (principal cells or interneurons) however remain to be elucidated.

It is thus important to discuss whether this time point (of grid cell emergence and functionality) also denotes a main turning point in the functional maturation of place cell firing. At first glance this does not seem to be the case. Most of the properties of place cells develop in a gradual way and do not show a sharp and abrupt improvement/change around this age (see Figure III-14, Figure III-15, Figure III-16 and Figure III-17).

However, a closer look at some of the results presented in this thesis does seem to indicate some major changes at this time point in the animals' development.

First, there is the abrupt decrease of the average number of firing fields per place cell between P20-21 and P22-27 (see Figure III-16B). In adult animals most place cells in dorsal CA1 only have one firing field (Fenton et al., 2008; Jung et al., 1994; Muller et al., 1987; O'Keefe, 2007) and for an ideal cognitive spatial representation each building block (i.e. place cell) should unambiguously signal a certain location in an environment. The emergence of grid cell input onto place cells might therefore be responsible for this abrupt change in the number of firing fields, as theoretical models exist that use the input of several grid cells onto a place cell to result in unitary firing fields of the latter (Blair et al., 2008; Solstad et al., 2006).

Second, the abrupt change at around the age of weaning in disappearance of the dependence of place cell stability from field-to-wall distance (see Figure III-18). To investigate whether the emergence of grid cell input is indeed responsible for the absence of this effect in post-weanling rat pups and adult animals, experiments in adult rats could be one approach. Such experiments could employ a selective ablation of direct grid cell input onto CA1 place cells, which should induce a similar dependence of place cell stability on field-to-wall distance in adult animals.

IV.5 Sensory integration of place cells in developing rat pups

One initial hypothesis of this thesis was that place cells in young animals might not be configural as in adults (O'Keefe and Conway, 1978), and anchored predominately to certain individual local cues (esp. olfactory cues) inside the recording environment. This was based on the developmental trajectories of the sensory systems of rats. On the behavioural level olfaction is the first modality to appear, becoming functional much earlier than all other senses (if gustation is neglected) (Altman and Sudarshan, 1975; Cornwell-Jones and Sobrian, 1977; Rudy and Cheatle, 1977). Vision only appears around P15 and it is not before at least P40-45 when this modality is more or less fully functional (Fagiolini et al., 1994; Moye and Rudy, 1985; Prévost et al., 2010). Because visual acuity only reaches adult-like levels around P40-45 (Fagiolini et al., 1994), one can only conclude that at this time point developing rats should be able to perceive distal extra-maze visual cues to a similar extent as adult animals. At younger ages it is unclear if rat pups can perceive these stimuli at all, and therefore might not use them for orienting themselves inside the recording environment.

Did this work find evidence for this initial hypothesis? The answer is a clear 'no', since neither the stability nor the spatiality of place cells in young animals were influenced by an absence of single local cues in the recording environment. However, in several cases an effect on place cell spatiality was present only in the adult animals, suggesting a 'delayed' emergence of an influence of these cues on place cell firing. One possibility is that changes in place cell firing reflect a novelty signal, and that this signal can be triggered more easily in adult rats than in pups.

The following discussion will first address the issue of the spatial tuning of place cells within a single trial and then the separate problem of how this tuning is maintained across recording sessions where only parts of the original sensory input were present.

IV.5.1 Development of spatial tuning

IV.5.1.1 Place cells in young animals only show a mild reduction in quality of spatial tuning in novel environments

From previous work it is known that place cell quality in novel environments in adult animals undergoes some experience-dependent improvement when compared to highly familiar environments (Brun et al., 2008a; Cacucci et al., 2007; Frank et al., 2004; Wilson and McNaughton, 1993). That is, during the first exposure to an environment, the spatial tuning of place cells is reduced. This reduction in the spatial tuning of place cells in a novel environment was also found for adult controls in this thesis, the effect being strongest in the absence of visual cues (see Figure III-36 and Figure III-39). In rat pups however, this effect was largely absent in a novel environment. Unfortunately no data is available for post-weanling rat pups for these probe trials (for the six cells recorded for the 'novel light' probe a reduction in all three parameters measuring place cell quality is present during the probe trial: spatial information: ca. -0.77 bits/spike, spatial coherence: ca. -0.17, intra-trial correlation: ca. -0.12). This seems to indicate that place cells in adult animals become much more tightly bound to the sensory cues after repeated exposures to an environment, while this either takes much longer or is generally weakened in pre-weanling rat pups. This result is very interesting, because environmental novelty in adult rats is associated with i) a reduction in the spatial

tuning of place cells and ii) remapping of the place cell representation (Bostock et al., 1991; Leutgeb et al., 2004; Lever et al., 2002). These effects seem to be dissociated in pre-weanling animals as here remapping is present in the novel environments (see Figure III-37 and Figure III-40), while the spatial tuning remains largely unaffected (see Figure III-36 and Figure III-39).

IV.5.1.2 A novelty induced reduction of the spatial tuning of place cells in very young animals (<P18-19) is also absent in sensory manipulation trials

One of the most surprising findings of this thesis was the near absence of an effect on the spatial tuning of place cells in very young animals when certain parts of the familiar environment were manipulated (see Figure III-24, Figure III-27, Figure III-30, Figure III-33, Figure III-36 and Figure III-39). Irrespective of whether the walls (see Figure III-24) or the floor (see Figure III-27) were replaced for visually identical copies or whether visual input was absent (see Figure III-33), place cell quality is generally not strongly affected in animals aged between P14-17. A complete independence of place cell firing on sensory information in these young animals would imply that they only use self-motion based information and/or e.g. geometrical information like the distance to one or several boundaries. Although there is evidence for dead reckoning in animals as young as P16 (Loewen et al., 2005), this is unlikely for several reasons. Sole reliance on path integration quickly accumulates errors (Etienne and Jeffery, 2004; Etienne et al., 1988; Save et al., 2001), and it is safe to assume that rat pups should not be superior in their path integrating abilities compared to adult rats. Furthermore, a reduction in inter-trial stability is present during the 'familiar dark' as well as the 'new floor & new walls' probe in animals aged P14-17 (see Figure III-31 and Figure III-34), indicating a general influence of sensory information on place cells at these age bins.

It is more likely that place cells in these very young animals do not yet show the above mentioned experience-dependent improvement of the spatial tuning of place cells in familiar environments compared to novel environments (Brun et al., 2008a; Cacucci et al., 2007; Frank et al., 2004; Wilson and McNaughton, 1993) (see section IV.5.1.1). In this thesis, place cells recorded from adult animals indeed showed a significant reduction in place cell quality in all of the above mentioned probe trials, except when the walls were replaced. Because all sensory cues inside the familiar environment were kept as constant as possible throughout the experiments (see section II.4), even a change of only a subset of those cues might induce this effect of novelty on place cell quality in adult animals. This interpretation is further supported by the lack of an effect on spatial tuning during recordings in a novel environment under light conditions in animals younger than P20 (see Figure III-36). One general reason for this effect might be a difference in the abilities of detecting environmental novelty in very young animals.

In adult rats acetylcholine (ACh) release in the hippocampal system is believed to be associated with environmental novelty (Acquas et al., 1996; Barry et al., 2012b). The hippocampal ACh-system undergoes substantial postnatal maturation (Aznavour et al., 2005; Nyakas et al., 1994). While around P16 the laminar distribution of ACh-fibres in the hippocampus resembles that of adult animals, the fibre density staining for ACh-esterase doubles between P10-20 (Nyakas et al., 1994) and the distribution of ACh-varicosities in the hippocampus reaches adult levels only at P32 (Aznavour et al., 2005). This immaturity in the hippocampal ACh-system might thus be responsible for the absence of this novelty-induced effect on the spatial tuning of place cells in pre-weanling animals. In fact, when probed with a global change of the local intra-maze cues (‘new floor & new walls’), even place cells recorded from very young animals show some reductions in place cell quality (see Figure III-30). Hence, a novelty signal should generally be present in the hippocampus of developing rats, but the degree of its strength will vary to that of adult animals under some conditions (e.g. weaker in ‘new floor’ probe), while in others it might of comparable degrees or even stronger (e.g. ‘new floor & new walls’ probe) .

IV.5.1.3 An effect of the absence/change of sensory cues of different modalities on the spatial tuning of place cells emerges at similar time points in development

The sensory systems of new born rats emerge in the following order: olfaction, gustation, touch, hearing, vision (hearing and touch emerge in parallel) (Cornwell-Jones and Sobrian, 1977; Crowley and Hepp-Raymond, 1966; Fagiolini et al., 1994; Grant et al., 2012; Hyson and Rudy, 1987, 1984; Landers and Zeigler, 2006; Moye and Rudy, 1985; Prévost et al., 2010; Rudy and Cheatle, 1977; Uziel et al., 1981). At least for olfactory/tactile and visual cues there is good evidence they can exert some control on place cell firing in adult animals (Hill and Best, 1981; Markus et al., 1994; Quirk et al., 1990; Save et al., 2000). This was also found in the adult control group in this thesis. When the olfactory cues on the floor were changed, place cell quality is reduced compared to the familiar environment (see Figure III-27 and Figure III-30).

Intuitively one might think that for developing rat pups the influence of these cues becomes apparent in the order of their functional emergence. However, this result was not conclusively found, although there seems to be some evidence for a corresponding hierarchy.

The absence of visual cues as well as a change of all local olfactory/tactile intra-maze cues ('new floor & new walls') both show a clear effect on the spatial tuning of place cells in pre-weanling animals at the same time in development (see Figure III-30 and Figure III-33). Around P18-19 the first clear effects are observable, and in post-weanling rat pups a reduction similar to the one of adult place cells is present in both probe trials, especially for the 'familiar dark' probe. Just as for adult animals the impact of a removal of visual cues was stronger in a novel environment than in a highly familiar one for rat pups (see Figure III-39). For animals younger than P18 a change of all local olfactory/tactile cues seemed to have a slightly stronger impact on place cell quality than an absence of visual cues (compare Figure III-30 and Figure III-33),

which might reflect the stronger immaturity of the visual system compared to those for smell and touch at this time point in development.

As discussed previously (see section IV.5.1.2) the absence of a reduction in the spatial tuning of place cells when changing local cues on the environment floor (see Figure III-27) may reflect differences in the abilities of pre-weanling animals to detect environmental novelty.

These results seem to indicate that i) place cells are inherently tuned to use visual cues even at a time point when the visual system is far from being mature and that ii) very early in development local olfactory/tactile cues might play a stronger role for place cell firing than at later stages. It would certainly be interesting to investigate at which point in development a rotation of a polarising visual cue (e.g. cue card) will result in a corresponding rotation of place field locations as it is the case for adult place cells (Bostock et al., 1991; Fenton et al., 2000; Hetherington and Shapiro, 1997; Muller and Kubie, 1987; O'Keefe and Speakman, 1987). Although such a cue should probably be located inside the recording environment for very young rat pups, from the results in this thesis it might well be that this effect is not present until P18.

In the real world visual landmarks are usually temporally and spatially very stable, while olfactory cues are volatile and will change over time. From an ethological point of view this might be one of the reasons for the very early influence of vision on place cell firing.

IV.5.2 Stability of place cells across probe trials indicates their inherently configural properties

As previously described (see section IV.1.3.1) the responses of place cells during the sensory manipulation trials were remarkably similar to those of adult animals (see Figure III-25, Figure III-28, Figure III-31, Figure III-34, Figure III-37 and Figure III-40).

IV.5.2.1 Even in young animals the hippocampus only requires part of the original sensory input to maintain a stable representation

Replacing the floor, the walls or removing visual input in probe trials demonstrated that place cells at each of the age bins only require parts of the original sensory input to re-activate/maintain a previously formed spatial representation. This effect is well described for adult animals (Fenton et al., 2000; O'Keefe and Conway, 1978; Quirk et al., 1990; Save et al., 2000).

Since replacing the walls had no statistically significant effect on place cell representations of the familiar environment at any age bin (see Figure III-25), it seems that local cues on the walls, if any, only play a minor role in determining the location specific firing of place cells.

Replacing the floor lead to a moderate reduction in place cell stability for post-weanling animals and adult controls (see Figure III-28). These results are in line with a study by Save et al. (2000) who also found some control of olfactory cues on the environment floor for place cell stability. By contrast, for pre-weanling rat pups no clear reduction in stability (except at P18-19) could be observed. This result was rather counterintuitive, given the time line of the development of the rat's sensory systems and the observed effect in adult rats. One possible

explanation might be the fact, that in adult and post-weanling animals, only those place cells remap that are bound specifically to an individual cue on the floor (e.g. urine smell), an effect which could be absent in young animals. For adult animals it is known that place cells in CA1 are not necessarily configural, but can be bound to a single specific cue (Hetherington and Shapiro, 1997). But since reports of single cue control of place cells is very scant in the literature (except for polarising visual cues), and since in the above cited study (Hetherington and Shapiro, 1997) the cue of interest was a visual one, this explanation seems rather unlikely.

More likely seems to be the interpretation that adult rats are better at detecting environmental novelty, due to their fully developed sensory systems. As described previously (see section IV.5.1.2) the immaturity in the hippocampal ACh-system might thus be reflected by a decreased level of remapping in place cells for pre-weanling animals in the 'new floor' probe. In fact, when probed with a global change in the local intra-maze cues ('new floor & new walls'), the majority of place cells show remapping in these young animals, presumably because that induces a strong enough novelty signal (see IV.5.2.2 and Figure III-31).

The results of the 'familiar dark' probe clearly show that visual input per se plays a similar role for place cell stability in very young rats as in adult animals (see Figure III-34). At all age bins, removal of visual input lead to a moderate reduction in place cell stability. The fact that most cells are unaffected by the removal of visual input, corroborates previous findings that the absence of this input can be compensated for by place cells by other local cues, presumably those on the environment floor as well as information about self-motion (Quirk et al., 1990; Save et al., 2000). On the other hand, it also might indicate that a complete absence of visual information produces a strong enough novelty signal in the developing hippocampus for a small amount of remapping to occur (since in this probe the complete input of one modality is absent) and furthermore supports the inherent tuning of place cells to visual stimuli (see section IV.5.1.3).

The early influence of vision on place cell stability might be surprising given the rather late postnatal functional maturation of the visual system, which is not fully adult-like until P40-45 (Fagiolini et al., 1994). However, behavioural results from cued-platform experiments in the water maze indicate that rat pups can solve this version of the task between P17-19 (Akers et al., 2011; Brown and Whishaw, 2000; Rudy et al., 1987), and pre-weanling animals can learn an association of a visual stimulus and an electric shock at P17-18 (Moye and Rudy, 1985). This indicates that although their visual acuity is still rather poor at this stage, rat pups can nonetheless use visual information to some extent. Furthermore, detection of light is probably ethologically quite relevant to survival for developing animals in the wild, and already at P6 negative phototaxis can be observed in rat pups (Routtenberg et al., 1978).

IV.5.2.2 Replacing both floor and walls indicates distal visual cues might not be perceived by pre-weanling animals

When both floor and walls from the familiar environment were replaced for replicas, remapping of place cells occurred at all age bins (see Figure III-31). For adult controls the amount of remapping was very similar to what was observed when only the floor was replaced (compare Figure III-28 and Figure III-31). For these animals it thus seems that mainly the change of local cues on the floor of the familiar environment are responsible for this effect.

Despite the absence of an interaction effect of age and environment for this probe trial, it seems that there is a trend of stronger remapping in pre-weanling animals (average reduction in inter-trial stability: P16-17: 0.39±0.06, P18-19: 0.54±0.05, P20-21: 0.49±0.07, P22-27: 0.32±0.08, adults: 0.23±0.03) (see Figure III-29). This seems to indicate two things. First, when probed with a strong change in the local intra-maze cues, strong remapping is induced in pre-weanling animals. Second, pre-weanling animals probably do not use the distal extra-maze

cues (which are stable) for anchoring place cells. The latter could explain why in older animals a trend for less remapping of the place cell representation is present and furthermore why there seems to be less strong remapping during the 'familiar dark' probe in pre-weanling animals (average reduction in place cell stability for 'familiar dark' probe: P16-17: 0.17±0.06, P18-19: 0.23±0.06, P20-21: 0.12±0.05, P22-27: 0.13±0.09, adults: 0.2±0.04) (compare Figure III-31 and Figure III-34). The former is a good indicator that generally sensory information is integrated in place cells of young rats, albeit a stronger global change has to happen for remapping to occur compared to post-weanling and adult animals. Changing all local intra-maze cues in the familiar environment might thus produce a strong novelty signal even in the hippocampus of young animals for global remapping of the place cell ensemble to occur under such conditions. This result also indicates that early in development local olfactory/tactile cues might have a stronger relevance for place cell firing compared to visual cues than at later time points (see section IV.5.1.3).

One point to note is the strong reduction in intra-trial correlation for the 'new floor & new walls' probe, an effect which is not present when the floor or the walls were replaced in single probe trials (see Figure III-30C) or during the 'novel light' probe (see Figure III-36C). This could indicate that novelty in a familiar location has a different effect on place cell firing compared to novelty in an unfamiliar location, especially for pre-weanling pups. In other words, the difference between a mismatch of expectation (novelty in familiar place) and an unexplored place (novel environment) might be responsible for this effect.

IV.5.2.3 General conclusions from the analysis of place cell stability in sensory manipulation trials

The above discussed results highlight that place cells have inherently configural properties even in very young animals. Irrespective of whether vision was absent or individual parts of the local intra-maze cues were changed, the majority of place cells can maintain a more or less stable firing under such conditions at every age tested. Local cues on the environment walls seem to generally only play a minor role for place cell stability as do local cues on the floor for pre-weanling animals. The absence of a statistically significant effect on place cell stability of replacing only the floor in pre-weanling animals might be explained by a developmental difference in the 'strength' of a novelty signal in the hippocampus, e.g. through the ACh-system. If however animals were probed with a strong change in sensory input in the familiar environment ('familiar dark' and 'new floor & new walls') place cells do remap in very young animals. For these probes the local intra-maze cues seemed to have a slightly stronger impact on place cell stability in rat pups compared to those of adult rats. This might either indicate an early developmental dominance of place cell control of local intra-maze olfactory/tactile cues over visual ones or that a novelty/mismatch signal in the developing hippocampus is stronger during the 'new floor & new walls' probe trial. In the latter probe probably all sensory cues in the environment change for pre-weanling animals while in the 'familiar dark' the local olfactory/tactile cues are still present.

The results of the latter two probe trials also indicate the double dissociation between stability and spatiality in pre-weanling animals as it seems to be present in the novel environments. That is, an absence of a strong effect on the spatial tuning accompanied by a more or less strong remapping of the place cell ensemble. Later in development these two effects seem to always occur in parallel. The reasons for this remain unclear, but it might be that the former

effect reflects a general maturation of the sensory systems and their inputs onto place cells, while the latter is due to maturation of the dentate-to-CA3 axis, hypothesised to be the neural systems involved in pattern separation and completion processes. The results of this thesis would then indicate that pattern completion and separation mature earlier.

IV.5.2.4 Novel environments induce novel spatial representations at every age bin

The recordings in the novel environments constitute an important control for assessing the animals' ability to detect drastic changes from a familiar environment. From recordings in CA1 from adult animals it is known that the hippocampus usually forms a novel spatial representation when environments are sufficiently different in their properties, like e.g. in location, shape and/or colour (Bostock et al., 1991; Leutgeb et al., 2005a, 2004; Lever et al., 2002; Wills et al., 2005). It is noteworthy that especially for CA1, even rather drastic changes can lead to only partial remapping of the place cell representation between two different environments under certain conditions (e.g. certain overlap between environments or insufficient amount of experience across two environments) (Bostock et al., 1991; Leutgeb et al., 2004; Lever et al., 2002). This was also found for place cells recorded from CA1 in adult animals in this thesis (see e.g. cell 3 for adults in Figure III-35). Although the majority of place cells remap, a smaller subset keeps their firing fields from the familiar environment, presumably because the shape of the two environments was the same (all square) and also some of the distal cues were overlapping, since the recordings were not conducted in a cue controlled environment.

For pre-weanling rat pups, place cell responses were overall very similar to those of adult animals. Irrespective of age, a more or less similar amount of remapping was present. This

indicates that even very young animals already possess the ability to differentiate between different environments, based on the differences in local/distal sensory cues.

One interesting difference to adult animals is the lesser number of place cells that show exclusive activity in either the familiar or the novel context (see Table III-18 and Table III-21). The percentages for adult animals approximate previously published proportions, although they seems to be slightly higher than expected from these studies (Guzowski et al., 1999; Thompson and Best, 1989). This could indicate that in adult animals a stronger orthogonalisation of the spatial representations in CA1 occurs between different environments compared to young rats, or in other words a more pronounced pattern separation process.

IV.6 Knock-on effects

The reason for a much more frequent occurrence of 'knock-on' effects of certain probe trials in pre-weanling rat pups compared to post-weanling and adult animals can only be speculated about. It might simply reflect a reduced stability of the place cell representation of a familiar environment in young animals over time. One could imagine that place cells in CA1 are more plastic in rat pups, i.e. more prone to permanently change their firing fields in response to a change in input. Equally likely would be a less coherent response of individual place cells in terms of the ensemble firing after certain parts of the sensory cues were manipulated in probe trials. In other words, while older animals fully re-activate the representation of the familiar environment after a probe trial, individual place cells in younger animals are more prone to e.g. keep an altered firing field location, thereby slightly changing the place cell representation of the familiar environment. This could reflect immaturity in the CA3 network. If one assumes attractor-type properties for CA3 in adult rats (pattern completion) (Rolls and Kesner, 2006), a less coherent response of the place cell ensemble in CA3 in developing animals might result in similar effects upstream in CA1.

It is noteworthy that after lesions of the direct entorhinal input to CA1 in adult animals, a reduction in inter-trial correlation across two trials in a familiar environment was present in the lesion group (Brun et al., 2008a). These trials were either interleaved by recording trials in a novel environment or an interval of one hour. The authors present some example rate maps, of which a subset showed remapping across the two trials in the familiar environment (see example cells 1-3 in Figure 6A in Bruns et al. (2008a)). Unfortunately, the authors did not analyse this effect in detail and thus it remains speculation that grid cell firing (i.e. its emergence in developing animals) might be responsible for a much increased stability of the place cell representation in older animals.

IV.7 General concluding remarks on place cell and behavioural development

Overall the results for the development of place cell firing found in this thesis seem to match well with the development of the behavioural repertoire of rat pups.

With the emergence and maturation of sensory systems (Cornwell-Jones and Sobrian, 1977; Fagiolini et al., 1994; Grant et al., 2012; Hyson and Rudy, 1984; Landers and Zeigler, 2006; Moye and Rudy, 1985; Prévost et al., 2010; Rudy and Cheatle, 1977; Uziel et al., 1981) and motor behaviour (Altman and Sudarshan, 1975; Bolles and Wood, 1964; Thiels et al., 1990; Westerga and Gramsbergen, 1990) during the third and fourth week, rat pups spend less and less time with the litter huddle (Loewen et al., 2005), start to leave the nest for increasing periods of time (Gerrish and Alberts, 1996; Loewen et al., 2005), and start to feed independently (Bolles and Wood, 1964; Gerrish and Alberts, 1996; Thiels et al., 1990). From an ethological perspective this requires functional brain systems for navigation to food sources and back to a safe shelter. In other words rat pups need to develop the ability to form stable representations of environments and/or trajectories. This thesis added to previous research which described the general development of place cell properties, in that also the integration of sensory information undergoes a rapid maturation during this time period. Furthermore the ability of rat pups to solve different behavioural paradigms like the water maze (Akers and Hamilton, 2007; Akers et al., 2009, 2007; Brown and Kraemer, 1997; Brown and Whishaw, 2000; Rudy et al., 1987; Schenk, 1985) or T-maze (Douglas et al., 1973; Green and Stanton, 1989), that in adult rats require normal hippocampal functioning (Dudchenko et al., 2000; Johnson et al., 1977; Morris et al., 1982; Riedel et al., 1999), is known to emerge during the fourth week. It thus seems that the maturation of place cell firing during this time period parallels the development on the behavioural level and further supports the idea of a cognitive

spatial map in the hippocampus, as proposed by O'Keefe and Nadel in the late 1970's (O'Keefe and Nadel, 1978a). Another point to consider is the question why most of the above mentioned hippocampus-dependent behavioural tasks emerge around P21 and not earlier. The results of this thesis demonstrated the inherent configural properties of place cells, even early in development. Furthermore, at all ages containing rat pup data similar proportions of significantly spatially tuned place cells were present. But, since place cell stability was only correlated to distance from environmental boundaries in pre-weanling animals, the disappearance of this effect around P21 (together with the emergence of grid cells firing) might be an important factor for the ability of young rat pups to solve a task like e.g. the water maze.

One last important point to consider is the question whether the functional maturation of place cell properties reflects developmental processes intrinsic to the hippocampal circuits, or whether they rather depend on maturational processes in primary sensory systems, or whether they reflect a combination of both. In keeping with this, one could interpret the decreased 'spatiality' of place cells in early development in several ways. On the one hand it seems plausible that this is only due to the immaturity of the sensory systems and throughout development the sensory input to the hippocampus simply becomes more adult-like leading to more spatially defined firing fields. On the other hand it would also be possible that the functional maturation of place cells only reflects developmental changes inside the hippocampal system, like e.g. changes in synaptic efficacy or postnatal changes of intrinsic or input/output connections.

Unfortunately it is very difficult to differentiate between these processes and this thesis cannot give a conclusive answer as to which might be the dominating factor. The author personally favours a combination of both, since clearly neither all sensory systems nor the hippocampal system itself is functionally adult-like by the end of the fourth week of a rat's life.

One strong argument in favour of the development of place cells being a process intrinsic to the hippocampus is the finding that some place cells appear essentially adult-like as early as P14: if the sensory input is available to drive adult-like firing at these ages, in theory it could also be available to all cells in the hippocampus. This argument is further strengthened by one finding of this thesis, that such precocious place cells are not based on simple sensory cue responses, but rather on a higher-order integration of multi-modal sensory information.

Experiments trying to dissociate between the two processes will be technically very challenging, if not impossible, as development in both the sensory systems and the hippocampus might influence each other, rendering it impossible to keep a sensory system in an immature state (through lesions or pharmacological manipulations) while observing the functional maturation of the hippocampus under such conditions. If e.g. one would surgically induce blindness in rat pups (before the eyes actually open) and then went on and recorded place cells in such treated animals, compensation effects might ensue, thus not telling us anything about how visual information gets integrated in normal animals. One possible approach would be to develop non-spatial behavioural tests that could assess the level of 'maturity' of individual sensory systems. Then one could correlate the results of these tests with place cell properties in individual animals. This approach would obviously still not reveal the locus of the functional maturation inside the brain, but could be a first step to more generally understand what constitutes the main driving force of these maturational processes.

However, there is one effect found in this thesis which is likely to be solely intrinsic to the hippocampal system: the dependence of place cell stability on the field-to-wall distance in pre-weanling animals, an effect which is absent in older rats. The relatively abrupt development of place field stability in the centre of the environment, from P22 onwards, is not reflected by any abrupt improvement in sensory systems at this age, suggesting a developmental process intrinsic to the hippocampal formation. In adult rats boundary sensitive neurons have only

been identified in the subiculum (Lever et al., 2009) and entorhinal cortex (Solstad et al., 2008) and there is some preliminary evidence from our (BVCs in subiculum) (personal communication by Francesca Cacucci) as well as the Moser laboratory (border cells in MEC) (Bjerknes et al., 2012), that these cells can also be identified in those regions in very young animals.

IV.8 Conclusions

The results of this thesis present compelling evidence that place cells in pre-weanling rats are inherently configural, as it is known for adult animals. Although in pre-weanling rat pups the sensory systems are still maturing on the functional level, their place cells are remarkably stable when certain parts of the local sensory cues of a familiar environment were removed/changed. Furthermore, place cells early in development seem to be driven by similar types of sensory modalities as those of adult animals, with a slight bias for local olfactory/tactile intra-maze cues over visual ones. Thus, despite the fact that there is a slow functional maturation of place cell spatial tuning and stability, these results indicate that even at the youngest ages, the hippocampus does contain a higher-order representation of space, consistent with the hypothesis that it is a Kantian synthetic *a priori* system. In other words even in very young animals the hippocampal system seems to be inherently tuned to integrate different sensory cues and already possesses the ability to re-activate a representation even if certain sensory cues in the environment were changed.

In ethological terms it is probably of high importance for developing rat pups that there is a rapid maturation of the ability of the hippocampus to form a stable representation of an environment, even in the face of local changes of the sensory input. One interesting point on the behavioural level refers to the emergence of the ability of rat pups to solve different hippocampus-dependent tasks like e.g. the water maze around P21. As shown in this thesis the hippocampus of rats should already contain a fair proportion of stable place cells at this age, which raises the question why the ability to solve this task emerges at such a 'late' time point in development. One possibility is that before P22 only those place cells that are located near an environmental boundary (in case of the water maze this would be the pool walls) are sufficiently stable and thus animals can only learn this task once this dependence (of place cell

stability and field-to-wall distance) disappears. In fact, when trained in a down-scaled pool low levels of spatial learning are already present in rats trained between P17-19 (Carman and Mactutus, 2001).

The results of this thesis strengthened the understanding on how the hippocampal system develops on a functional level. However, several open key questions remain that have to be addressed in future experiments:

It could be shown in this work, as well as in previously published studies (Langston et al., 2010; Scott et al., 2011; Wills et al., 2010), that place cells undergo a strong improvement of their location-specific firing both in terms of quality and stability. It is still unclear whether this can be observed on the single cell level when following the properties of the same place cells over multiple days/weeks. As previously mentioned an imaging approach (e.g. as in Ziv et al. (2013)) would be a possible way to address this question.

As far as the development of sensory integration is concerned the obvious question that remains is what exactly the underpinnings of the maturational process are: Are these developmental changes inside the sensory systems, the hippocampal system or both? This question will be very hard to address as it is extremely difficult to distinguish between the two. One possible approach would be to develop behavioural tests (that are tailored for the response repertoire of rat pups) assessing the properties of different sensory systems across different animals and then correlate possible differences between animals with differences in their respective place cell properties. This would obviously require recordings of large place cell ensembles in individual animals which is why an imaging approach should probably be taken to address this question.

Finally, a better understanding of what makes boundaries so important for place cell stability in pre-weanling animals is also of high significance. On the one hand one would want to try to

observe the emergence of boundary sensitive cells in the hippocampal-parahippocampal system. From the results in this thesis one would expect an early emergence concurrent to the one of place cells as well as a very rapid functional maturation of these cells. On the other hand one could try and re-establish to some extent the 'pre-weanling state' of the hippocampus without functional grid cell input by removing this input from the hippocampus proper. If indeed the emergence of grid cell input to the hippocampus around P21 is responsible for the subsequent absence of this effect in post-weanling and adult animals, one would expect to possibly see this effect in adult animals under such conditions.

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VI Appendix

VI.1 Abbreviations

AC: autocorrelation

ACh: acetylcholine

ADN: anterior dorsal nucleus of the thalamus

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

BVC: boundary vector cells

CA: Cornu Ammonis

CR: Cajal-Retzius

DG: dentate gyrus

Exx: embryonic day xx

EC: entorhinal cortex

EEG: Electroencephalogram

GABA: γ-aminobutyric acid

GDP: giant depolarising potential

HCP: hippocampus proper

HD cell: head direction cell

HF: hippocampal formation

IPSP: inhibitory postsynaptic potential

LEC: lateral entorhinal cortex

LFP: local field potential

LIA: large irregular activity

LTD: long-term depression

LTP: long-term potentiation

MEC: medial entorhinal cortex

NMDA: *N*-methyl-D-aspartate

OC1B: binocular portion of V1

Pxx: postnatal day xx

PaS: parasubiculum

PER: perirhinal cortex

PFA: paraformaldehyde

PHF: parahippocampal formation

POR: postrhinal cortex

PoS: postsubiculum

PrS: presubiculum

REM: rapid eye movement

rs: rhinal sulcus

SEM: standard error of the mean

SIA: small irregular activity

SPW: sharp waves

SUB: subiculum

V1: primary visual cortex